# BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

## IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/34807 A2

(51) International Patent Classification[7]: C12N 15/12, C07K 14/705, 14/435, C12Q 1/68, C12N 5/10, C07K 16/28

(21) International Application Number: PCT/US00/41521

(22) International Filing Date: 25 October 2000 (25.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

| | | |
|---|---|---|
| 60/161,564 | 26 October 1999 (26.10.1999) | US |
| Not furnished | 24 October 2000 (24.10.2000) | US |

(71) Applicants and
(72) Inventors: BULLA, Lee, A., Jr. [US/US]; 6455 U.S. Hwy. 377, Tioga, TX 76271 (US). CANDAS, Mehmet [US/US]; 13859 Peyton Drive, Dallas, TX 75240 (US).

(74) Agent: VAN DYKE, Raymond; Jenkens & Gilchrist, P.C., Suite 3200, 1445 Ross Avenue, Dallas, TX 75202-2799 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *Without international search report and to be republished upon receipt of that report.*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/34807 A2

(54) Title: *PECTINOPHORA GOSSYPIELLA* (PINK BOLLWORM) *BACILLUS THURINGIENSIS* TOXIN RECEPTOR BT-$R_2$

(57) Abstract: A cDNA encoding a 200 kD receptor, BT-$R_2$, from the pink boll worm, *Pectinophora gossypiella*, that binds specifically to a *Bacillus thuringiensis* toxin has been cloned, sequenced and characterized. The minimum toxin binding fragment has been identified. The BT-$R_2$ cDNA permits the analysis of receptors in pink boll worm and other insects that affect crop growth and development, as well as, design assays for the cytotoxicity and binding affinity of potential pesticides. The clone and other methods described herein, permit the manipulation of natural and/or introduced homologous receptors and, thus, to specifically destroy organisms, tissues and/or cells of the target host.

# *PECTINOPHORA GOSSYPIELLA* (PINK BOLLWORM) *BACILLUS THURINGIENSIS* TOXIN RECEPTOR BT-$R_2$

## CROSS-REFERENCES TO RELATED APPLICATIONS

This Application for Patent claims the benefit of priority from, and hereby incorporates by reference the entire disclosure of, co-pending U.S. Provisional Application for Patent Serial No. 60/161,564 filed October 26, 1999.

## TECHNICAL FIELD OF THE INVENTION

This invention generally relates to receptors for *Bacillus thuringiensis* (BT) toxin and thus to pesticides able to bind the receptor, and to ameliorating pesticide resistance. In particular, the invention relates to recombinant DNA and expression systems for a novel receptor and receptor elements from *Pectinophora gossypiella*, the pink bollworm.

## BACKGROUND OF THE INVENTION

Without limiting the scope of the invention, its background is described in connection with uses of *Bacillus thuringiensis* toxins as cotton insect biocidal agents, as an example. Cotton insect pests reduced yields by almost 10% across the US in 1998. Insect damage reduced the overall cotton yield by more than 1.7 million bales and produced a financial loss of about $1.224 billion. One group in particular, the bollworm/budworm complex was the most damaging causing a 2.7% loss. The pink bollworm, *Pectinophora gossypiella* Saunders ("*PBW*"), is a lepidopteran insect that causes severe damage to cotton and is the most destructive pest of cotton worldwide.

*Bacillus thuringiensis* is a gram positive, sporeforming bacterium that forms a parasporal crystal which contains insecticidal toxins (Bulla et al., *Crit. Rev. Microbiol.* (1980) 8: 147-204; Höfte and Whiteley, *Microbiol. Rev.* (1989) 53: 242. The effect of the toxin is mediated through binding to specific receptors on the apical brush border of the midgut microvillae (BBMV) of susceptible insects.

Biological control of cotton pests using *B. thuringiensis* formulations and transgenic plants has been in use for a number of years and is growing rapidly. Recently, transgenic cotton plants carrying the toxin genes of BT have been developed and sold commercially. Such transgenic plants have a high degree of resistance to the pink bollworm (Schnepf et al., *Microbiol. Mol. Biol. Rev.* (1998) 62: 775). However, the introduction of any new insecticide into a pest management program immediately initiates a selection process for individuals that are resistant to the pesticide. As the use of transgenic crops expressing BT toxin increases, insect resistance is expected to become more widespread. Increased tolerance for BT toxins in several species of insects has been reported by several investigators while laboratory selection experiments have shown that the use of BT toxin formulations and transgenic plants can provoke the development of resistance in the pink bollworm (Bartlett, et al., *Beltwide Cotton Conference* (1995) 2: 766).

Concerns that BT toxin formulations or transgenic plants expressing the toxin genes may evoke emergence of either resistant or tolerant strains of insects has made the search for a better understanding of the interaction between the BT toxin proteins and their respective insect receptors a matter of considerable economic importance.

In U.S. Patent No. 5,693,491, the present inventors disclosed the purification and cDNA cloning of a *B. thuringiensis* toxin receptor BT-$R_1$ from larvae of the tobacco hornworm *Manduca sexta* (*M. Sexta*). Recently, two BT toxin receptors have been identified, purified and cloned from the silkworm, *Bombyx mori* (Nagamatsu et al., *Biosci. Biotechnol. Biochem.* (1998) 62: 727).

Heretofore in this field, there has been no structural information concerning the structure and function of BT toxin receptor of the major cotton insect pest, *P. gossypiella*. Furthermore, to the inventors' knowledge, the minimum binding fragment encoding a consensus binding domain for BT toxin on the BT receptor has not yet been identified. Isolation of the minimum binding fragment could permit cloning and structural characterization of important yet uncharacterized BT toxin receptors from other insects of worldwide economic importance such as *P. gossypiella*.

## SUMMARY OF THE INVENTION

The present invention provides information and materials for isolation and expression of novel BT crystal toxin receptors, herein referred to as Cry toxin receptors. Generally, the invention provides structural and functional characterization of a novel lepidopteran BT toxin receptor, herein referred to as BT-$R_2$.

A cDNA that encodes an alternative glycoprotein receptor from the pink bollworm that binds specifically to a *B. thuringiensis* toxin has been cloned, sequenced and characterized. The BT-$R_2$ cDNA permits the analysis of receptors in pink bollworm and other insects and organisms that affect crop growth and development, as well as the design of assays for the cytotoxicity and

binding affinity of potential pesticides. The clone and other methods described herein, permit the manipulation of natural and/or introduced homologous receptors and, thus, to specifically destroy organisms, tissues and/or cells of the target host, including insects resistant to toxins of *B. thuringiensis*.

The invention further provides purified and cloned cDNA encoding a 200 kD receptor for the Cry1A toxins of the pink bollworm, *P. gossypiella*. An advantage of this invention is the identification of the minimum binding fragment encoding the toxin binding domain on the BT toxin receptor. Another advantage of this invention is the provision of methodologies for cloning and structural characterization of presently unknown BT receptors. Furthermore, this invention provides methods and materials for identification and design of effective toxin binding receptors for use in combating emergence of toxin resistance. Also, this invention may be used to generate transgenic organisms expressing toxin receptors.

## BRIEF DESCRIPTION OF THE DRAWINGS

A more complete understanding of the method and apparatus of the present invention may be obtained by reference to the following Detailed Description when taken in conjunction with the accompanying Drawings wherein:

FIGURES 1A-B show the nucleotide sequence cDNA encoding the BT-$R_2$ protein from *P.gossypiella* (SEQ ID NO:1);

FIGURES 2A and 2B show the amino acid sequence of BT-R2 protein from *P. gossypiella* (SEQ ID NO: 2). Arrows indicate the start site of the putative cadherin domains **CR1 - CR12**, **SIG** = signal sequence (double underline); **MPD** = membrane proximal domain; **CYT** = cytoplasmic region. The transmembrane region is

underlined and bold. The leucine zipper motif **LZ** is underlined. Ñ residues denote putative N-glycosylation sites. The minimum binding fragment **MBF** (aa 1269-1367) is also double underlined;

Figure 3A is a graph showing the binding results of Cry1A toxins on *P.gossypiella* larvae brush border membrane vesicles prepared from midgut epithelial cells;

Figure 3B is a graph showing the toxicity results of Cry1A toxins on *P. gossypiella* larvae and BBMV;

Figure 4 is a map of the structure of the pink bollworm (PBW) BT-$R_2$ cDNAs, including truncations PBW-1210-1439, PBW-1269-1439, PBW-1367-1496, and PBW-1210-1367 (the minimum binding fragment). The binding of proteins expressed from each clone to Cry1A toxin was identified by (+) for binding and (-) for non-binding; and

FIGURES 5A-C illustrate an alignment of the silk worm (top), the tobacco hornworm (middle), and the pink bollworm (bottom) Cry toxin receptors. Perfectly conserved residues are boxed.

## DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EXEMPLARY EMBODIMENTS

The present invention will now be described more fully hereinafter with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

ABBREVIATIONS AND DEFINITIONS

The following abbreviations are used throughout this application: bp - base pairs; BT - *Bacillus thuringiensis* or *B. thuringiensis*; BT-$R_x$ - BT toxin receptor of type x; BBMV - brush border of the membrane vesicles; cDNA - complementary DNA; Cry toxin - parasporal crystalline toxin of *BT*; IEF - immunoelectrophoresis; kb - kilobase or kilo base pairs; kD - kilodaltons; $K_d$ - dissociation constant; $LC_{50}$ - lethal concentration resulting in a 50% mortality; PBW - pink bollworm, *Pectinophora gossypiella* or *P. gossypiella*; PCR - polymerase chain reaction; RACE - Rapid Amplification of cDNA Ends; RT - reverse transcriptase; SW - silkworm (*Bombyx mori* or *B. mori*); THW - tobacco hornworm (*Manduca sexta* or *M. sexta*); and UTR - untranslated region.

The term "x% homology" refers to the extent to which two nucleic acid or protein sequences are identical as determined by BLAST homology alignment as described by T.A. Tatusova & T.L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS MICROBIOL LETT. **174**:247-250 and using the following parameters: Program (blastn) or (blastp) as appropriate; matrix (OBLOSUM62), reward for match (1); penalty for mismatch (-2); open gap (5) and extension gap (2) penalties; gap x- drop off (50); Expect (10); word size (11); filter (off). An example of a web based two sequence alignment program using these parameters is found at http://www.ncbi.nlm.nih.gov/gorf/bl2.html.

The invention thus includes nucleic acid or protein sequences that are highly similar to the sequences of the present invention, and include sequences of 80, 85, 90, 95 and 98% similarity to the sequences described herein.

The invention also includes nucleic acid sequences that can be isolated from genomic or cDNA libraries or prepared synthetically, and that hybridize under high stringency to the entire length of a 400 nucleotide probe derived from the nucleic acid sequences described herein under. High stringency is defined as including a final wash of 0.2X SSC at a temperature of 60°C. Under the calculation:

Eff Tm = 81.5 + 16.6(log M [Na+]) + 0.41(%G+C) - 0.72(% formamide)

the percentage allowable mismatch of a gene with 50% GC under these conditions is estimated to be about 12%.

The nucleic acid and protein sequences described herein are listed for convenience as follows:

| SEQ ID Nos.: | DNA and Protein Sequences |
|---|---|
| SEQ ID NO: 1 | BT-$R_2$ cDNA sequence from *P. gossypiella* (Figure 1) |
| SEQ ID NO: 2 | BT-$R_2$ protein sequence for *P. gossypiella* (Figure 2) |

| SEQ ID Nos.: | Primer Sequences | Primer Name |
|---|---|---|
| SEQ ID NO: 3 | 5' CAN ATH CGN GCN CAN GAY GGN GG 3' | BTR 1209U |
| SEQ ID NO: 4 | 5' TTG TAC ACS GCW GGS ATW TCC AC 3' | BTR 1355U |
| SEQ ID NO: 5 | 5' NAC YTG RTC RAT RTT RCA NGT CAT 3' | BTR 1486D |
| SEQ ID NO: 6 | 5' NCC DAT NAG RTC NGA RTC RTT NGA 3' | BTR 1657D |
| SEQ ID NO: 7 | 5' TAG GTT GTA TCC TCA GTA TGA GGA 3' | PBW-BTR GSP-1 |
| SEQ ID NO: 8 | '5' CCA GAG TGG AGT CCA CCG CCA TA 3' | PBW-BTR GSP-2 |

| | | |
|---|---|---|
| SEQ ID NO: 9 | 5' CTG AGT AAG TGT TAT CTT GAA AG 3' | PBW-BTR GSP-3 |
| SEQ ID NO: 10 | 5' CAN ATH CGN GCN CAN GAY GGN GG 3' | BTR 1209U |
| SEQ ID NO: 11 | 5' GAT AGC GGC CCC AGG AAC CAA CAA ACA GG 3' | PBW-BTR GSP-4 |
| SEQ ID NO: 12 | 5' AGT GCG AGT GCT TTG AAT CTG TGA 3' | PBW-B'IR P2U |
| SEQ ID NO: 13 | 5' GTC TCT TCT CAC CGT CAC TGT CAC T 3' | PBW-BTR P5U |
| SEQ ID NO: 14 | 5' GCA TGC TGG CAG TAG GTT GTA TC 3' | PBW-BTR P6D |
| SEQ ID NO: 15 | 5' GGC CAC GCG TCG ACT AGT AC 3' | (AUAP) |
| SEQ ID NO: 16 | 5' GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT TTT TTT T 3' | (AP) |

N = A, C, T, or G; H = A, T, or C; B = T, C, or G; D = A, T, or G; V = A, C, or G; R = A or G; Y = C or T; M = A or C; K = T or G; S = C or G; W = A or T

More particularly, the studies described herein were targeted toward the identification, cloning and characterization of novel Cry toxin receptors. One embodiment was directed to characterization and isolation of the heretofore unidentified Cry toxin receptor of the pink bollworm, *P. gossypiella*, hereinafter referred to as "PBW".

In order to identify and isolate the Cry toxin receptor of the PBW, toxicity was determined for five different Cry proteins (Cry1Aa, Cry1Ab, Cry1Ac, Cry3A and Cry11A) against neonate PBW larvae. It was determined that the lepidopteran-specific toxins (Cry1Aa, Cry1Ab and Cry1Ac) showed high toxicity toward PBW larvae with a $LC_{50}$ ranging from 25-45 ng/cm$^3$ of insect diet, while the coleopteran specific (Cry3A) or the dipteran specific (Cry11A) toxins did not exhibit any detectable toxicity up to 2000 ng/cm$^3$ (Figure 3).

The binding of the three lepidopteran-specific Cry1A toxins (Cry1Aa, Cry1Ab and Cry1Ac) to the BBMV of *P. gossypiella* was characterized in detail. Ligand blot experiments showed that proteins of 120 kD bind only the Cry1Ac toxin whereas a 200 kD protein binds to Cry1Aa, Cry1Ab and Cry1Ac toxins. It is now known that the 120 kD protein is a heat shock protein, although its relation to the Cry toxin effect is not understood.

In the case of the 175 kD cadherin-like Cry1Aa binding protein from *Bombyx mori*, $^{125}$I -labeled Cry1Aa binding was eliminated by the presence of unlabeled Cry1Aa, but additional band(s) of approximately 110 kD, identified by $^{125}$I-Cry1Aa ligand blots, failed to demonstrate a detectable degree of competition. Thus, it was determined that *P. gossypiella*, like *M. sexta* and *B. mori*, contains *both* high-affinity and low-affinity binding proteins for at least one Cry1A toxin and that the 200 kDa protein from PBW is a common binding protein for the lepidopteran-specific Cry1A toxins.

The detailed mechanism of the Cry1A toxin interaction with the midgut BBMV of the pink bollworm was determined. The equilibrium dissociation constants ($K_d$) calculated from the homologous competition assays (Figs. 3A and 3B) are 16.5, 12.4 and 12.8 nM and the concentrations of binding sites are 3.7, 3.6 and 8.6 pmol/mg, for Cry1Aa, Cry1Ab and Cry1Ac, respectively. The Hill Coefficients for the three Cry1A toxins are between 0.6 and 0.8 for BBMV binding proteins (Figure 3A), indicating that there is negative cooperativity in the binding of these toxins to the binding site(s) in the BBMV. Binding of the Cry1A toxins to BBMV proteins was specific and saturable. The toxin amount required for saturation of 460 μg of BBMV proteins was in the following order: Cry1Ac>Cry1Aa>Cry1Ab.

Immunoprecipitation of BBMV proteins with anti-Cry1Ab antiserum and subsequent ligand blotting with $^{125}$I-Cry1Ab toxin also showed binding of the toxin to an approximately 200 kD protein. The 200 kD protein is a single protein as shown by 2D-gel analysis (data not shown). A comparison between the 210 kD binding protein from *M. sexta* with a pI ~ 4.3 and the 200 kD binding protein from P. *gossypiella* (pI - 4.1) revealed that both proteins have almost the same pI. It was determined that the 200 kD PBW protein had some cross-reactivity with polyclonal antisera against the *M. sexta* BT-$R_1$ 210 kD protein.

In order to clone the PBW BT-$R_2$ gene, fully degenerate primers were designed based on the conserved amino acid sequences between that of the two receptors, tobacco hornworm ("THW") BT-$R_1$ and silkworm ("SW") BT-R175. The primer locations were designed to include or exclude a sequence thought by the present inventors to encode a region in the extracellular domain critical to toxin binding, herein after "READ" signature sequence. Hereinafter this binding fragment of the DNA sequence will be referred to as the "signature" region.

Three clones were obtained, PBW-421 (aa 1367-1496), PBW-866 (aa 1210-1496) and PBW-1373 (aa 1210-1675), which have about 50 % nucleotide and about 60% amino acid sequence similarity to both THW BT-$R_1$ and SW BT-R175. The 421 bp and 866 bp clones encode proteins of about 21 and 32 kD, respectively. Although both expressed proteins cross-reacted with THW BT-$R_1$ polyclonal antisera, the 32 kD protein, but not the 21 kD protein, was shown to bind Cry1Ab toxin specifically with high affinity. The estimated $K_d$ value is about 17 nM, which is similar to the $K_d$ value obtained for BBMV. Similarly, an internal fragment from the PBW-866 clone did not bind toxin, but did cross-react with BT-$R_1$ antibodies. This data demonstrates that

recognition by anti-BT-$R_2$ antibodies is insufficient to define a functional toxin receptor.

In order to obtain a cDNA sequence encoding the full-length receptor, the 5' and 3' ends of the PBW BT-$R_2$ receptor were first obtained using 5' and 3' RACE reactions followed by cloning of the full-length receptor cDNA using gene specific primers from the 5' and 3' UTR. The full-length cDNA clone (SEQ ID NO: 1) has an open reading frame of 1729 amino acids (SEQ ID NO:2), with a deduced molecular weight of 194 kD and a calculated pI value of 4.1, which is similar to the value determined by 2-D gel analysis.

The protein consists of three domains: extracellular, transmembrane and cytoplasmic. The protein sequence contains two hydrophobic regions, one at the amino terminus, characteristic of a signal peptide and one near the COOH-terminus (amino acids 1575-1600) that probably forms a transmembrane domain. The extracellular domain contains 12 cadherin-like motifs, in addition to, a membrane proximal region that contains two leucine zipper motifs. Eleven consensus sites for N-linked glycosylation are present in the extracellular region, which may account for the difference in apparent molecular mass between the native protein and the calculated mass.

Based on the results discussed above, it would be apparent to one of ordinary skill in the art that variances in receptor sequences or in toxin binding affinities or in receptor expression may render different levels of toxin susceptibility or resistance. Furthermore, the receptor of the present invention may be used to generate transgenic organisms by methods well known in the art.

To investigate the mode of action of BT toxin, a mammalian heterologous cell culture system was chosen for several reasons. First, BT Cry1A toxins have shown

no toxic effect on any mammalian cell lines studied to date. This characteristic is in contrast to most available insect cell lines, which exhibit variable degrees of sensitivity to toxin (Kwa et al., 1998). Second, the use of a mammalian cell would allow the determination of whether the receptor, independent of any associated protein in an insect cell line, would mediate toxicity.

When introduced into mammalian COS-7 cells, the cloned cDNA expressed BT-$R_2$ that was detected by western blot analysis using BT-$R_2$ antisera. The expressed receptor was displayed on the cell surface and detected with polyclonal antibodies raised against *M. sexta* BT-$R_1$. These results suggest that the protein expressed by the PBW BT-$R_2$ cDNA is similar to the natural protein found in the insect midgut.

The possibility of using COS-7 mammalian cells transfected with a receptor for BT toxins as a model system for assessing the cytotoxicity of the Cry1A toxin was determined. The surface receptor clearly was able to bind to the Cry1Ab toxin, which was detected by immunofluorescent labeling using Cry1Ab antibodies (data not shown). These results indicate that the binding site of the receptor must assume its native conformation. Significantly, intensively labeled vesicles in the methanol fixed transfected COS-7 cells were observed when the cells were incubated with BT-$R_1$ antiserum (data not shown). This observation indicates that vesicles, which form normally in the cell endocytosis/exocytosis pathway, contain the BT-$R_2$ proteins. In addition, this result shows that the receptor is not only expressed on the cell surface, like its native counter part in the insect midgut, but also is recycled normally by the cell.

Microscopy of the transfected COS-7 cells treated with Cry1Ab toxins for various times demonstrated

significant cytopathological patterns. The cytopathological changes observed under the fluorescent microscope included disruption of the plasma membrane, cell swelling, disintegration and death of the cells. The symptoms were obtained in the presence of 0.6 μg/ml Cry1Ab for 2 hr. In contrast, no cytopathological effects were revealed for cells transfected with vector alone and subsequently treated with toxin. Clearly, there is a distinct correlation between toxin binding to the surface receptor and toxicity to the cells.

The cytological appearance and ultrastructure of the midgut cells of *M. sexta* and other lepidopteran larvae, after intoxication with preparations of BT, have been reported extensively by several authors (Bravo et al., 1992). Histopathological studies on *M. sexta* midgut demonstrated pathological behavior for Cry1A on midgut epithelial cells (columnar cells) (Midhoe et al., 1999). These investigators demonstrated that the epithelial cells of the midgut swell shortly after ingestion of the BT toxin. Eventually, the epithelial cells burst and released their cytoplasmic contents into the midgut lumen.

The present observations on the intoxicated transfected COS-7 cells are in complete agreement with these reports, which demonstrates that the toxin acts similarly in both systems. Furthermore, it should be apparent to one of ordinary skill in the art that cells expressing transfected molecules of the BT toxin receptor as well as cells expressing a natural form of the receptor may be used to asses the level of cytotoxicity and mode of action of toxins.

Lepidopteran insects generally express high molecular weight binding proteins for the Cry1A toxins that range in size from 160 to 220 kD (Martinez-Ramirez 1994; Vadlamudi et al.; 1993, Oddouet al., 1993; Nagamatsu et al., 1998a; Ihara et al., 1998). Two of

these proteins, in addition to the 200 kD pink bollworm receptor, have been cloned and sequenced: the BT-R$_1$ 210 kD cadherin-related receptor from *M. sexta* (Vadlamudi et al., 1995) and the 175 kD cadherin-related from *B. mori* (Nagamatsu et al., 1998a). Interestingly, these two proteins have 60-70% identity and 80% similarity between themselves.

*P. gossypiella* expresses a high-affinity and a low-affinity binding protein for at least one Cry1A toxin, Cry1Ac. The high-affinity receptor is a cadherin-related protein with a large molecular mass. One of the most important conserved regions may be the signature sequence. The signature sequence contains the sequence (READ), which is believed to be responsible for toxin binding due to the presence of two negatively charged amino acids that bind to two arginines in the toxin binding site. Supporting evidence comes from the immunoblot analysis for clones PBW-866, which contains the proposed signature sequence, and PBW-421, which does not include the signature sequence. To further define the minimum binding fragment, truncation peptides were tested for their ability to bind toxin (Figure 4). The minimum binding fragment contains the "READ" signature sequence and consists of amino acids 1269 to 1367.

The information provided herein is necessary for understanding the molecular biology of the toxin receptor in the pink bollworm and to engineer more effective toxins in terms of longer persistence in the field, higher toxicity, and preclusion of resistance development. This information will facilitate understanding of Cry toxin receptor interactions in other economically important insect crop pests.

EXAMPLE 1 SPECIFICITY OF PURIFIED TOXINS

Recombinant protoxins Cry1Aa, Cry1Ab, and Cry1Ac (*Bacillus* Genetic Stock Center, Ohio State University) were prepared from *E. coli* JM-103 and trypsinized essentially as described by Lee et al. *J. Biol. Chem.* (1992) 267: 3115. In addition, the soluble trypsinized 60 kD toxins were subjected to FPLC NaCl salt gradient purification over an HR-5/5 Mono-Q anion exchange column (PHARMACIA™) prior to quantitation, radio-iodination, and use in bioassays. Cry3A crystal protein from *B. thuringiensis* subsp. *tenebrionis* was solubilized in 3.3 M NaBr and treated with papain, and the resulting 67 kD toxin was purified by the method of Li et al. *Nature* (1991) 353: 815. The 65 kD Cry11A toxin was isolated from *B. thuringiensis* subsp. *israelensis* via solubilization as described by Chilcott et al. *J. Gen. Micro* (1988) **134**: 1551 and further purified by anion-exchange FPLC. All toxin protein quantitations were performed using the bicinchoninic acid method (PIERCE CHEMICAL™) with Bovine Serum Albumin (BSA, Fraction V) as a standard.

Pink bollworms were obtained from the USDA PINK BOLLWORM REARING FACILITY™ (PBWRF, Phoenix, AZ). An artificial diet was obtained from SOUTHLAND PRODUCTS INC.™, Lake Village, AR. The diet was reconstituted in boiling water and cooled to 55°C. Each Cry toxin was thoroughly mixed in the warm liquid diet and bioassay cups were filled with 20 ml of diet. After cooling and drying, 10 neonate larvae were placed in each cup and the cups were immediately capped. The method of Watson, et al., *Beltwide Cotton Conference*, Memphis, Tenn. (1995) was used to determine the toxicity of trypsin-activated toxins against first-instar larvae of *P. gossypiella*. Generally, four replicates of six cups were prepared for each dose. Cups were incubated at 30° C for 21 days, the length of

time necessary for more than 95% of normal *P. gossypiella* to reach pupation. At the end of 21 days, the diet cups were examined and the numbers of larvae and numbers of pupae or adults in each cup were recorded.

The specific toxicities of purified Cry1Aa, Cry1Ab, Cry1Ac, Cry3A and Cry 2A tested using neonate *P. gossypiella* larvae are shown in Figure 3B. It was determined that all three Cry1A toxins are highly toxic, with $LC_{50}$ values ranging from 25-45 ng/cm$^3$ of artificial diet. Cry3A (considered toxic to coleopteran or beetle insects) and Cry IIA (considered toxic to dipteran insects, especially mosquitoes) were not toxic to *P. gossypiella* larvae at the highest concentrations tested (2000 ng/cm$^3$).

EXAMPLE 2 CHARACTERIZATION OF THE BT-$R_2$ RECEPTOR

Early fourth-instar larvae were kept on ice for 1 hr and midguts were surgically removed from the larvae. BBMW were prepared from midgut tissues by the differential magnesium precipitation method of Wolfersberger, et al., *Comp. Biochem. Physiol.* (1987) 86A: 30, in the presence of protease inhibitors (5 mg/ml pepstatin, antipain, aprotonin, leupeptin, 1 mM PMSF, and 5 mM benzamidine). The final pellet was resuspended in buffer A (300 mM mannitol, 5 mM EGTA, and 17 mM Tris-HCl, pH 7.5) containing the protease inhibitors, flash frozen in liquid nitrogen, and stored at -85°C.

Cry toxins were radioiodinated using the chloramine T method (Hunter and Greenwood, *Nature* (1962) **194**: 495, with $^{125}$I-Na (NEN DUPONT ™). Ten μg of toxin were mixed with 5 μl of $^{125}$I-Na (0.5 mCi) in 100 μl of $NaHPO_4$ buffer (0.5 M, pH 7.4) with 25 μl of Chloramine T (4 mg/ml). The reaction mixture was agitated for 20-25 seconds at 23°C and the reaction was

stopped by adding 50 μl of $Na_2S_2O_5$ (4.4 mg/ml). Free iodine was removed by gel filtration on an EXCELLULOSE ™ desalting column (PIERCE ™) equilibrated with PBS containing 10 mg/ml BSA.

TOXIN BINDING ASSAYS.

Both homologous and heterologous competition inhibition binding assays were performed as described by Keeton and Bulla (1997). A total of 25 μg of BBMV were incubated with 1.2 nM $^{125}$I-Cry1Ac toxin in the presence of increasing concentrations (0-1000 nM) of the appropriate unlabeled homologous toxin (Cry1Ac) or heterologous toxins (Cry1Aa, Cry1Ab, Cry3A, and Cry11A). Incubations were in 100 μl of binding buffer (PBS/0.2% BSA) at 25°C for 30 min. Radiolabeled and unlabeled toxins were mixed together before adding them to the BBMV. Unbound toxins were separated from BBMV-bound toxin by centrifugation at 14,000 x g for 10 min. The pellet containing bound toxin was washed three times in ice cold binding buffer by gentle vortexing and radioactivity in the final pellet was measured using a BECKMAN GAMMA 5500 ™ counter. Binding data were analyzed by the PRISM ™ program (GRAPHPAD SOFTWARE INC.™, San Diego).

Competition inhibition binding of $^{125}$ I-Cry1Ac toxin to *P. gossypiella* was carried out in the presence of increasing concentrations of unlabeled Cry1Ac, Cry1Ab, Cry1Aa, Cry3A and Cry11A toxins. Homologous competition binding assays were performed with iodinated Cry1A toxins and various concentrations of the corresponding unlabeled toxin. The binding site concentration ($B_{max}$), and dissociation constant ($K_d$) of labeled toxins were calculated from three separate experiments. The equilibrium binding parameters were estimated by analyzing the data with the PRISM™ computer program.

RADIOLIGAND BLOTTING.

The two hundred μg of BBMW proteins were solubilized, separated by 7.5% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane as described by Francis and Bulla (1997). Blots were blocked with TBS (10 mM Tris-HCI and 0.9 % NaCl) containing 5% non-fat dry milk powder, 5% glycerol 0.5% Tween-20, and 0.025% sodium azide for 2 hr at 25°C. Blocking buffer was removed and membranes were incubated for 2 hr at 25°C in an equal volume of fresh blocking buffer containing 2 x $10^5$ cpm/ml (1-1.25 nM) of $^{125}$I-Cry1A toxins either in the presence or absence of unlabeled toxins. Finally, membranes were washed three times with fresh blocking buffer for 10 min each, rinsed once with TBS, dried, and exposed to Kodak X-ray film at -80° C.

To determine the specificity of binding to the 200 and 120 kD proteins, blots of PBW BBMV proteins was incubated with $^{125}$I-Cry1Ac toxin in the presence of increasing concentrations of unlabeled Cry1Ac toxin.

IMMUNOPRECIPITATION OF CRY1AB BINDING PROTEIN.

Immunoprecipitation was carried out according to Vadlamudi, et al. (1993). Twenty five μl of Cry 1Ab antiserum were added to 1 ml of protein A-Sepharose CL-4B equilibrated in washing buffer (1% Nonidet P-40, 6 mM EDTA, 50 mM Tris-HCl and 250 mM NaCl) and mixed for 1 hr at 4°C. After washing the blot three times with washing buffer, 700 μg of Cry 1Ab toxin were added and the mixture were incubated for an additional 1 hr at 4°C and washed again three times with washing buffer. Pink bollworm BBMV proteins (6 mg) were solubilized in washing buffer containing 1% NP-40 and protease inhibitors (10 μg/ml pepstatin, antipain, aprotonin and leupeptin; 5 mM iodoacetamide; and 1 mM PMSF).

Unsolubilized proteins were removed by centrifugation. Solubilized proteins were filtered through a 0.45 μm filter, added to 1 ml of Sepharose-protein A beads linked to Cry1Ab toxins, and the sample was stirred gently for 1 hr at 4°C. Sepharose beads were centrifuged and washed four times with washing buffer containing 0.25% NP-40 and 0.02% SDS. The toxin-binding protein complex was dissociated by heating in Laemmli (1970) sample buffer and the binding proteins were Coomassie stained and detected by ligand blotting with $^{125}$I-Cry1Ab and Western blot using Cry1Ab antiserum.

IMMUNODETECTION OF PINK BOLLWORM CRY1A RECEPTOR.

Immunoprecipitated proteins were transferred to a PVDF membrane, blocked with 5% nonfat dry milk in PBS buffer and incubated at 4°C overnight in the same blocking buffer containing 10 μg/ml of Cry1Ab. Unbound toxin was washed with PBS. Antibodies raised in rabbits against the 60 kD Cry1Ab toxin were diluted 1:1000 and hybridized to the membrane for 2 hr at 25°C and the blot then was washed with PBS. Peroxidase-conjugated goat anti-rabbit IgG was diluted 1:3000 in TBS blocking buffer and hybridized to the membrane for 2 hr. The membrane then was washed extensively with PBS. Visualization of the bound toxin was accomplished using the Enhanced Chemiluminescence (ECL) Western blotting detection method (AMERSHAM™).

SOUTHERN BLOT ANALYSIS.

Forty μg of PvuH digested genomic DNA from *P. gossypiella* or *M. sexta* were separated on a 0.8 % 1X TBE-agarose gel and blotted onto a nylon membrane (BIO-RAD™, ZETA-PROBE GT™). The analysis was carried out according to Sambrook, et al. *Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor*

*Laboratory, N.Y.* (1989). The filter was hybridized with $^{32}$P-labeled, random primed, C-terminal of BT-$R_1$ cDNA (HincH fragment, 0.5 kb). Filter hybridization was carried out at 42°C for 21 hr in 50 % formamide, 5X Denhardt's reagent, 1M NaCl, 2% SDS, 50 mM Tris-HCl and 100 μg/ml of salmon sperm DNA. The filter was washed with 2X SSC, 0.5% SDS, then with 1X SSC, 0.5% SDS, then with 0.5X SSC, 0.5% SDS, followed by a fourth wash with 0.25X SSC, 0.5% SDS. Each wash was for 30 min at 42°C. Finally, the filter was rinsed in 2X SSC and exposed to Kodak X-ray film at -85°C.

ELECTROPHORETIC ELUTION OF PROTEINS.

Electrophoresis was performed in 1.5-mm-thick polyacrylamide slab gels using 7.5% acrylamide (pH 8.0). After SDS-PAGE, proteins were revealed as transparent bands with 4 M sodium acetate solution. The proteins were excised using a razor blade. Proteins in the gel strips were fixed in 50 % (v/v) methanol solution for 15 min and equilibrated twice in 0.125 M Tris-HCl buffer (pH 6.8) and 2% 2-mercaptoethanol for an additional 15 min. Equilibration of the gel strips in the above buffer with 1% (w/v) SDS was performed as described above. The equilibrated gel strips were inserted into a dialysis tube with a minimum amount of the buffer containing SDS (25 mM Tris, 190 mM glycine and 0.1% SDS). Electroelution was carried out essentially as described by Findlay (1990). A horizontal flat-bed mini-gel electrophoresis apparatus (BIO-RAD™) was used for electroelution at 50 V for 12 hr at 4°C. The buffer consisted of 25 mm Tris, 190 mM glycine and 0.1% SDS (pH 8.3). At the end of electrophoresis, the polarity of electrodes was changed for 30 sec to avoid adsorption of proteins onto the dialysis tubes. The buffer inside the dialysis tubes was collected and the

tubes were washed three times with a minimum volume of buffer. SDS was dialyzed out and protein was concentrated by using a CENTRICON-30 micro-concentrator (AMICON).

TWO-DIMENSIONAL GEL ELECTROPHORESIS.

Two-dimensional gel electrophoresis was performed according to the method of O'Farrell (1975). Isoelectric focusing was carried out in 2.0 mm (I.D.) glass tubes using 2.0% ampholines (pH 3.5-10; LKB/PHARMACIA™) for 9600 volt-hr. After equilibration for 10 min in buffer `O', tube gels were applied to the stacking gels on top of 8% acrylamide (pH 8.0) slab gels (14 x 14 cm). SDS slab gel electrophoresis was carried out for 4 hr at 12.5 mA. After electrophoresis, one gel was stained with Coomassie blue and the others were transblotted onto PVDF paper overnight at 200 mA (Vadlamudi et al., 1993). The PVDF paper was blocked with powdered milk solution, incubated with $^{125}$I-CrylAc or $^{125}$I-CrylAb and exposed to X-ray film at -85°C.

IDENTIFICATION AND RECOVERY OF cDNA ENCODING BT-$R_2$.

Total RNA was prepared from the midgut tissue of fourth instar larvae of the PBW by the guanidinium thiocyanate method (Chomczynki et al. *Analyt. Biochem.* (1987) **162**: 156). Poly (A+) RNA was isolated with the POLYATRACT MRNA ISOLATION SYSTEM™ (PROMEGA™). First strand cDNA was synthesized using oligo-(dT) and random hexamer primers and reverse transcriptase according to standard methodologies and used as the template for amplification by polymerase chain reaction (PCR) of desired mRNAs. Degenerate oligonucleotide primers were designed based on the conserved amnio acids between *M. sexta* BT-$R_1$ and *B. mori* BT-R175. Such primers were used to clone partial fragments of PBW BT-$R_2$.

For cloning of the PBW BT-$R_2$, RT-PCR was employed using fully degenerate oligonucleotide primers derived from a sequence in the membrane proximal domain conserved sequence between *M sexta* BT-$R_1$ and *B. mori* BT-R175. Primers BT-R-1355U and BT-R-1209U against BT-R-1486D were applied to PBW cDNA to amplify 421-bp and 866-bp fragments. The PCR products were resolved on 1.5% agarose, gel purified, cloned into a TA cloning vector (INVITROGEN™) and transformed into *E. coli* INV∝F. The presence and identity of the correct insert was confirmed with EcoR1 digestion and DNA sequencing. The PBW-886 clone was found to contain the nucleotide sequence found in clone PBW-421. In addition, primer 1209U against 1657D was used to clone a 1373-bp fragment (PBW-1373), which represents most of the membrane proximal domain and the cytoplasmic domain. Clone PBW-287 (aa 1346-1438) is a 287 bp internal fragment from 866-bp clone and was cloned using gene specific primers P5 and P6.

Based on the sequence obtained from the partial clones, sense and antisense primers were used to clone the 3' and 5' ends of the PBW BT-$R_2$ clone by the 5' and 3' RACE system according to the manufacturer's instructions (GIBCO BRL™). The 5' end was amplified using gene-specific antisense primers GSP1, GSP2 and GSP3 against ABRIDGED UNIVERSAL AMPLIFICATION PRIMER™ (AUAP™) provided in the kit. The 3' end was amplified using gene primer GSP4 against AUAP™. The PCR product of the predicted size was isolated and subcloned into TA cloning vector pCR2.1 (INVITROGEN™) and transferred into *E. coli* INV∝F. For recombinant protein expression in *E. coli*, or COS7 cells, the coding sequences for the RT-PCR clones or the full length PBW-BT-$R_2$ clone were recloned into the pET30 or pcDNA3.1 expression vectors and transformed into BL21 (DE3) LysS (NOVAGEN™) or COS7 mammalian cells. The *E. coli* cultures were

induced using a 1 mM final concentration of IPTG for 3 hr.

The full length PBW BT-$R_2$ (~5.5 kb; see sequence in Figure 1 SEQ ID NO:1) was ligated into the mammalian expression vector pcDNA3.1 (INVITROGEN™) and confirmed by DNA sequencing. The molecular mass of the deduced polypeptide is 194 kD with a pI of 4.1. The receptor has an open reading frame of 1729 amino acids (Figure 2) (SEQ ID NO: 2). The amino acid sequence contains a putative signal peptide of 23 amino acid residues, a transmembrane domain of 27 residues (aa 1578-1605) and a 124-residue cytoplasmic domain. In addition, the amino acid sequence contains 12 putative cadherin motifs, 11 putative N-glycosylation sites and two leucin zipper motifs at amino acid 1541-1562 and 1578-1600. The minimum toxin binding fragment is amino acids 1269 to 1367 (Figure 4).

When the protein homology is analyzed by BLASTP, as described under definitions above, the closest paralog in the GenBank nonredundant (nr) database is the *Bombyx mori* receptor at Acc. No. JE0128 with Identities = 1034/1708 (60%), Positives = 1266/1708 (73%), Gaps = 35/1708 (2%). The next closest species was *Manduca sexta* at Acc. No. AAB33758.1 with Identities = 871/1540 (56%), Positives = 1101/1540 (70%), Gaps = 22/1540 (1%). The nucleotide sequence showed no significant homologies.

The peptide homologies amongst these three species are shown in FIGURES 5A-C where perfectly conserved residues are boxed. Peptide fragments of the SBW sequence may be used to generate specific or nonspecific antibodies. Usually, it is recommended that at least 17 amino acid peptide fragments are used to generate antibodies, however, smaller peptides may also be antigenic and sufficiently complex to be unique. In particular, the carboxyl tail (aa 1677-end)

of the PBW sequence is unique to this species and can be used to generate PBW unique antibodies. Exemplary peptides that may be useful as antigens (numbered with respect to FIGURE 5, SEQ ID NO: 2) are shown as follows:

| PBW Unique Peptides | Common Peptides |
|---|---|
| aa 534-544 | aa 291-304 |
| aa 697-705 | aa 622-632 |
| aa 886-895 | aa 791-803 |
| aa 1055-1066 | aa 1621-1642 |
| aa 1321-1331 | |
| aa 1451-1461 | |
| aa 1516-1525 | |
| aa 1572-1582 | |
| aa 1677-1729 | |

IMMUNODETECTION OF THE EXPRESSED BT-$R_2$ PROTEINS.

Cell lysates from the induced BL21 (DE3) LysS bacterial cultures were electrophoresed and transferred to PVDF membranes. Filters were blocked at 4°C in 50 ml of blocking buffer containing 10 ug/ml of Cry1Ab toxin. Unbound toxin was removed by PBS. Rabbit primary antibodies for the THW was removed by PBS. Rabbit primary antibodies for the THW BT-R, extracellular domain or for the FPLC-purified Cry1Ab were diluted 1:1000 in 50 ml TBS blocking buffer. The filters were incubated for 2 hr with the antiserum and washed three times with the blocking buffer. Peroxidase-conjugated goat anti-rabbit IgG was diluted to 1:2000 and incubated with filters for 2 hr at 27°C and was developed with the enhanced chemoluminescence (ECL) detection system (AMERSHAM™).

MAMMALIAN EXPRESSION OF BT-$R_2$.

The PBW BT-$R_2$ cDNA cloned into pcDNA3.1, a mammalian expression vector (INVITROGEN™), was expressed in mammalian cells (COS-7 SV40 transformed African green monkey cells; ATCC CRL-1651) according to methods described by Keeton and Bulla, *Appl. Environ. Microbiol.* (1997) 63: 3419. COS-7 cells (4 x $10^5$/well) were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) on 12 mm cover slips placed in a 24-well plate.

COS-7 cells were transfected with the construct using the LIPOFECTAMIN PLUS REAGENT™ (GIBCO BRL™). The cells were incubated for two days at 37°C in DMEM medium containing 10% FBS in a humidified atmosphere of 10%$CO_2$. BT-$R_2$ was monitored by SDS-PAGE and immunoblotting with anti-BT-$R_1$ or antiCry1Ab antiserum. Surface expression was detected by immunofluorescence microscopy with the anti-BT-$R_2$ antibodies. The effects of BT toxin on the transfected cells were demonstrated by incubating the cells in the presence or absence of Cry1Ab toxin for 2 or 4 hr and monitoring the morphological changes by immunofluorescence microscopy using either anti-BT-$R_2$ or anti-Cry1Ab antibodies. Cell death is clearly demonstrated (not shown).

IMMUNOFLOURESCENCE MICROSCOPY.

COS-7 cells were grown on 12-mm glass coverslips in a 24-well plate. The cells were fixed and permeabilized either in cold methanol (-20°C) or 4% paraformaldhyde for 15 minutes at 27°C. Coverslips were rinsed three times with PBS and then blocked for 15 minutes with 1% BSA in PBS. Cells were incubated with primary antibody for 30 minutes at 27°C followed by rinsing and blocking as just described. The same

incubation and washing procedures were applied to secondary antibody. Antibodies were detected with TRITC goat anti-rabbit IgG. Coverslips were mounted in FLUROMOUNT G™ and viewed with an OLYMPUS™ microscope equipped with epi-fluorescence illumination and a 40X Apochromat lens. Photography was done with an OLYMPUS SPOT™ camera.

WESTERN BLOT ANALYSIS.

Transfected COS-7 cells were washed with cold PBS, lysed in lysis buffer (50 mM Tris/HCL, 1 mM EDTA, 10 μM leupeptin) and resuspended on ice for 10 minutes. Then, 4X sample buffer was added to the cells and heated at 95°C for 5 minutes. Lysates were subjected to electrophoresis through 7.5% SDS-PAGE, and proteins were electrophoretically transferred to a PVDF filter, blocked and incubated with either anti-BT-$R_1$, or anti-Cry1Ab antibodies.

RESULTS: IDENTIFICATION OF $^{125}$I-CRY1A BINDING PROTEINS.

BBMV proteins of *P. gossypiella* ranged in molecular size from greater than 205 kD to less than 25 kD (data not shown) as determined by SDS-PAGE. $^{125}$I-labeled Cry1Aa, Cry1Ab and Cry1Ac were used in ligand blots to identify which *P. gossypiella* BBMV proteins bind the respective toxins. Proteins that had been separated by SDS-PAGE were transferred to PVDF membranes and incubated with each radiolabeled-toxin separately. $^{125}$I-Cry1Aa, $^{125}$I-Cry1Ab and $^{125}$I-Cry1Ac bound to a protein of about 200 kD (data not shown). $^{125}$I-Cry1Ac bound also to a protein band at about 120 kD. Neither Cry1Aa nor Cry1Ab bound to the 120 kD protein. The binding patterns for all three toxins were the same under both reducing and nonreducing conditions (data not shown).

RESULTS: COMPETITION INHIBITION BINDING ASSAYS.

$^{125}$I-labeled Cry1Aa, Cry1 Ab and Cry1Ac were used in binding assays with *P. gossypiella* BBMV. Competition binding of $^{125}$I-Cry1Ac toxin to *P. gossypiella* was carried out in the presence of increasing concentrations of unlabeled Cry1Aa, Cry1Ab, Cry1Ac, Cry3A and Cry11A toxins. Fifty-percent inhibition of Cry1Ac binding was observed at 10 nM of unlabeled Cry1Ac, 100 nM unlabeled Cry1Aa and 100 nM of unlabeled Cry1Ab. At a concentration of 1000 nm, unlabeled Cry1Ac, Cry1Ab and Cry1Aa reduced binding of iodinated Cry1Ac by 95, 82 and 80%, respectively (data not shown). Neither Cry3A nor Cry11A toxin competed for the Cry1Ac toxin binding site.

Homologous competition binding assays were performed with iodinated Cry1A toxins and various concentrations of the corresponding unlabeled toxin Cry1Aa, Cry1Ab and Cry1Ac showed high binding affinity to BBW proteins (data not shown). Fifty-percent inhibition of binding of Cry1A toxins was observed at concentrations of approximately 10 nM of the corresponding unlabeled toxin. These data indicate that each of the three toxins binds specifically with high affinity. The binding site concentration, $B_{max}$, and the dissociation constant, $K_d$, of each toxin was calculated from the three separate homologous competition inhibition experiments by analyzing the data with the GRAPHAD computer program (Table 1). The $K_d$ values all were similar and in the low nM range whereas the $B_{max}$ for Cry1Ac was higher than Cry1Aa or Cry1Ab. The Hill coefficients for Cry1Aa, Cry1Ab and Cry1Ac were 0.65, 0.65, and 0.77, respectively, indicating a negative binding cooperativity for the toxins against the BBMV proteins. A single binding site model was indicated based on the nonlinear

regression analysis for both Cry1Aa and Cry1Ab. Significantly, Cry1Ac, the data was best accommodated by a two binding site model with high- and low-affinity binding sites.

RESULTS: SPECIFICITY OF $^{125}$I-CRY1AC TOXIN BINDING IN LIGAND BLOTS.

In view of the putative "two-binding site" model predicted for the Cry1Ac toxin, radioligand blots of *P. gossypiella* BBMV proteins were carried out with $^{125}$I-Cry1Ac toxin in the presence of increasing concentrations of unlabeled Cry1Ac toxin. Autoradiography of these blots revealed significant reduction in the intensity of the 200 kD band (data not shown). Indeed, it was undetectable at a Cry1Ac toxin concentration of 10 nM. In the case of the 120 kD band, however, there was virtually no reduction in the band intensity (data not shown) even at a Cry1Ac concentration of 1000 nM. In saturation binding assays, incubation of a fixed amount of each of the three $^{125}$I-labeled Cry1A toxins with increasing concentrations of BBMV showed that binding reached a saturation level in each case but that the level of Cry1Ac binding was substantially higher than those of Cry1Aa and Cry1Ab. Maximum saturable binding at 400 μg/ml of BBMV was approximately 0.35, 0.05 and 1.5 ng for Cry1Aa, Cry1Ab and Cry1Ac, respectively, which represents an approximately 30-fold difference in Cry1Ac binding compared to Cry1Ab, and, it is 4 fold higher for Cry1Ac compared to Cry1Aa (data not shown).

RESULTS: IMMUNOPRECIPITATION OF THE CRY1AB BINDING PROTEIN.

Immunoprecipitation experiments were performed using Cry1Ab, which has the highest binding affinity of the three toxins, to further examine the specificity of binding of the toxin to the 200 kD protein. BBMV proteins were solubilized in 1% NONIDET P-40™ and

immunoprecipitated with anti-toxin-protein A-Sepharose beads. The mixture of bound material was solubilized in SDS sample buffer containing 2-mercaptoethanol. Electrophoresis and staining of the gel with Coomassie blue revealed a protein of about 200 kDa, demonstrating selective precipitation of the 200 kD toxin-binding protein. Radioligand blotting with $^{125}$I-Cry1Ab showed a band of about 200 kDa (data not shown), indicating precipitation of the same binding protein as that identified in previous ligand blot experiments. Additionally, a Western blot (data not shown) of the immunoprecipitated protein using Cry1Ab and anti-Cry1Ab polyclonal antiserum confirmed the results of the radio-ligand blot (data not shown). The low-molecular weight bands at 60 and 52 kDa correspond to the Cry1Ab toxin and the heavy chain of IgG, respectively.

RESULTS: PURIFICATION OF THE BINDING PROTEINS.

To determine whether the 200 kD band contains more than one protein, the band was excised from a 7.5% SDS polyacrylamide gel, electroeluted, dialyzed and concentrated. The concentrated protein was analyzed by two-dimensional gel electrophoresis over a pH range of 3.5-10. The protein migrated as one spot with an estimated pI of 4.5 ± 0.2 and apparent molecular mass of 200 kDa. The purified 200 kD protein stained with Schiff's reagent (data not shown) indicating that the binding protein is glycosylated. The 200 kD IEF spot bound $^{125}$I -Cry1Ab (data not shown) corroborates the results from other immunoprecipitation studies.

RESULTS: SOUTHERN BLOT ANALYSIS.

To detect the presence of the Cry1A receptor in *P. gossypiella*, genomic DNA from both insects were hybridized against the cloned THW BT-$R_1$ cDNA and its 507-bp minimum binding fragment. The two probes bound

intensively to the PvuH fragment of *M. sexta* genomic DNA (data not shown). There was weak hybridization to the *P. gossypiella* DNA, however, using the minimum binding probe and none with the full-length BT-$R_1$ probe (data not shown). These results suggest that the minimum binding fragment from *M. sexta* shares a significant level of nucleotide similarity to the Cry1A binding receptor in *P. gossypiella*, more so than to the full-length BT-$R_1$ receptor.

RESULTS: IMMUNODETECTION OF NATIVE AND CLONED PBW BT-$R_2$ USING BT-$R_1$ ANTIBODIES.

To confirm the relatedness of the cloned PBW fragment to the THW BT-$R_1$ and its ability to bind toxin, it was subcloned into a pET30 expression vector. The native PBW BBMV proteins and the expressed proteins from clones PBW-287, -421 and -866 were resolved by SDS-PAGE, transferred to a PVDF membrane and incubated with either anti-BT-$R_1$ serum or Cry1Ab toxin followed by antiserum to the toxin. The results reveal that BBMV contain a 200 kD protein that interacts with THW BT-$R_1$ antiserum (data not shown). In addition, clones PBW-287, -421 and -866 which express proteins of about 15, 21 and 32 kD, respectively, also cross-reacted with BT-$R_1$ antiserum. The 32 kD clone, however, was the only protein to bind toxin, whereas no detectable binding was observed with the 21 kD protein (data not shown). These results confirm the sequence relatedness of PBW BT-$R_2$ to THW BT-$R_1$ and demonstrate that the 32 kD protein contains the toxin-binding site of the receptor.

RESULTS: SPECIFICITY OF TOXIN BINDING TO THE CLONED RECEPTOR.

The specificity and affinity of toxin binding to the receptor fragment (PBW-866) was determined using competition ligand blot analysis. The expressed 32 kD

protein was transferred to PVDF membranes and incubated with $^{125}$I-Cry1Ab in the absence or presence of increasing concentrations of unlabeled Cry1Ab toxin. Autoradiography revealed significant reduction in the intensity of the 32 kD band to an undetectable level in the presence of 500 nM unlabeled Cry1Ab toxin (data not shown). Bound $^{125}$I toxin was quantitated with a gamma counter and the BIO-RAD IMAGER™ analysis system was used to calculate the binding affinity of toxin to the expressed fragment. The binding affinity (~17nM) of the toxin was similar to the calculated value (Table 1) for BBMV. These results demonstrate that Cry1Ab binds specifically with high affinity to PBW BT-$R_2$ 866. Other truncation fragments were also tested, and it was determined that the minimum binding fragment consists of amino acids 1269 to 1367.

RESULTS: EXPRESSION OF PBW BT-$R_2$ IN COS-7 CELLS.

PBW BT-$R_2$ cDNA was subcloned into the mammalian expression vector pcDNA3.1 (INVITROGEN™) and transfected into COS-7 cells. Protein encoded by the PBW BT-$R_2$ cDNA was expressed as a membrane protein capable of binding Cry1Ab toxin. Membranes isolated from transiently transfected COS-7 cells were solubilized, electrophoresed, and immunoblotted either with Cry1Ab toxin and its antiserum or with BT-$R_1$ antiserum directly. The expressed 220 kD receptor bound Cry1Ab toxin and cross-reacted with BT-$R_1$ antiserum. No interaction to vector transfected cells was observed.

Expression of BT-$R_2$ receptor on the cell surface was shown by fixing the cells in methanol or paraformaldehyde and incubating first with anti-BT-$R_1$ serum, and then with TRITC IgG secondary antibodies. Transfected cells portrayed bright surfaces due to the binding of BT-$R_2$ antibodies to the cell surface clearly

showing that the PBW BT-$R_2$ receptor is expressed on the cell surface.

The surface-expressed PBW receptor binds toxin and kills the cells. Transfected cells were incubated with Cry1Ab toxin for 2 or 4 hr, washed, fixed and incubated first with anti-Cry1Ab antiserum, and then with TRITC IgG secondary antibodies. As shown by immunofluorescence microscopy, BT-$R_2$ expressing COS-7 cells bound the toxin, whereas cells transfected with vector alone did not show any surface binding of toxin. Incubation of cells expressing PBW BT-$R_2$ with toxin for 2 or 4 hr showed significant morphological changes which include loss of cell integrity, loss of cell cytoplasm and complete disintegration of the plasma membrane and cell death.

The prior cited and following references are incorporated by reference herein and are used to suport the invention disclosure:

J.S. Alexander et al., The role of cadherin endocytosis in endothelial barrier regulation: involvement of protein kinase C and actin-cadherin interactions, *Inflammation*, Vol. 22, pp. 419-433, 1998.

A.C. Bartlett, Resistance of the pink bollworm to B.T. transgenic cotton, *Beltwide Cotton Conf.*, Vol. 2, pp. 766-768, 1995.

P.C. Bolin et al., presented at the XXVIIth Annual Meeting of the society for Invertebrate Pathology, Cornell University, Ithaca, N.Y., 1995.

A. Bravo, Phylogenetic relationships of *Bacillus thuringiensis* delta-endotoxin family proteins and their functional domains, *J. Bacteriol.*, Vol. 179, pp. 2793-2801, 1997.

A. Bravo et al., Immunocytochemical Localization of *Bacillus thuringiensis* insecticidal crystal proteins in intoxicated insects, *J. Invertebr. Pathol.*, Vol. 60, pp. 237-246, 1992.

L.A. Bulla et al., Ultrastructure, physiology, and biochemistry of *Bacillus thuringiensis*, *Crit. Rev. Microbiol.*, Vol. 8, pp. 147-204, 1980.

J. Carroll et al., Analysis of the large aqueous pores produced by a *Bacillus thuringiensis* protein insecticide in *Manduca sexta* midgut-brush-border-membrane vesicles, *Eur. J. Biochem.*, Vol. 245, pp. 797-804, 1997.

N.C. Chilcott et al., Comparative toxicity of *Bacillus thuringiensis var. israelensis* crystal proteins *in vivo* and *in vitro*; *J. Gen. Micro.*, Vol. 134, pp. 2551-2558, 1988.

P. Chomczynki et al., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, *Analyt. Biochem.*, Vol. 162, pp. 156-159, 1987.

A.H. Dantzig et al., Association of intestinal peptide transport with a protein related to the cadherin superfamily, *Science*, Vol. 264, pp. 430-433, 1994.

M.L. Day et al., E-cadherin mediates aggregation-dependent survival of prostate and mammary epithelial cells through the retinoblastoma cell cycle control pathway, *J. Biol. Chem.*, Vol. 274, pp. 9656-9664, 1999.

R.A. De Maagd, Different Domains of *Bacillus thuringiensis* δ-endotoxins can bind to insect midgut

membrane proteins on ligand blots, *Appl. Environ. Microbiol.*, Vol. 62, pp. 2753-2757, 1996.

J.A. Dorsch et al., Isolation of the binding site in BT-$R_1$ from *Manduca sexta* for the insecticidal toxin of *Bacillus thuringiensis subsp. berliner*. In Preparation, 1999.

U. Estada et al., Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border of the cabbage looper, *Trichoplusia ni* (Hubner) (Lepidopteran: Noctuidae), and selection for resistance to one of the crystal proteins, *Appl. Environ. Microbiol.*, Vol. 60, pp. 3840-3846, 1994.

J. Ferre et al., Biochemistry and genetics of insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins, FEMS Microbiol. Lett., Vol. 132, pp. 1-7, 1995.

J.B. Findlay et al., Gel Electrophoresis of proteins-A Practical Approach, *Academic Pree, New York, 2nd ed.*, B.D. Hames and D. Rickwood (editors), pp. 83-89, 1990.

B.B. Finlay et al., Exploitation of mammalian host cell functions by bacterial pathogens, *Science*, vol. 276, pp. 718-725, 1997.

B.R. Francis et al., Further characterization of BT-$R_1$, the cadherin-like receptor for Cry1Ab toxin in tobacco hornworm (*Manduca sexta*) midguts, *Insect Biochem. Mol. Biol.*, Vol. 27, pp. 541-550, 1997.

S.F. Garczynski et al., Identification of putative insect brush border membrane-binding molecules specific to *Bacillus thuringiensis* δ-endotoxin by protein blot

analysis, *Appl. Environ. Microbiol.*, Vol. 57, pp. 2816-2820, 1991.

S.S. Gill et al., Identification, isolation, and cloning of a *Bacillus thuringiensis* Cry1Ac toxin-binding protein from the midgut of the lepidopteran insect *Heliothis virescens*, *J. Biol. Chem.*, Vol. 270, pp. 27277-27282, 1995.

S.S. Gill et al., The mode of action of *Bacillus thuringiensis* endotoxins, *Annu. Rev. Entomol.*, Vol. 37, pp615-636, 1992.

R. Gurezka et al., A heptad motif of leucine residues found in membrane proteins can drive self-assembly of artificial transmembrane segments, *J. Biol. Chem.*, Vol. 274, pp. 9265-9270, 1999.

J.L. Hermiston et al., In vivo analysis of cadherin function in the mouse intestinal epithelium: essential roles in adhesion, maintenance of differentiation, and regulation of programmed cell death, *J. Cell Biol.*, Vol. 129, pp. 489-506, 1995.

C. Hofmann et al., Binding of the delta endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly (*Pieris brassicae*), *Eur. J. Biochem.*, Vol. 173, pp. 85-91, 1998a.

C. Hofmann et al., Specificity of *Bacillus thuringiensis* delta-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts, *Proc. Natl. Acad. Sci. USA*, Vol. 85, pp. 7844-7848, 1988b.

H. Hofte et al., Insecticidal crystal proteins of *Bacillus thuringiensis*, *Microbiol. Rev.*, Vol. 53, pp. 242-255, 1989.

W. Hunter et al., Preparation of iodine-131 labeled human growth hormone of high specific activity, *Nature*, Vol. 194, pp. 495-496, 1962.

H. Ihara et al., Purification and partial amino acid sequences of the binding protein from *Bombyx mori* for Cry1Aa delta-endotoxin of *Bacillus thuringiensis*, *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.*, Vol. 120, pp. 197-204, 1998.

S.S. Kantak et al., E-cadherin regulates anchorage-independent growth and survival in oral squamous cell carcinoma cells, *J. Biol. Chem.*, Vol. 273, pp. 16953-16961, 1998.

T.P. Keeton et al., Ligand specificity and affinity of BT-$R_1$, the *Bacillus thuringiensis* Cry1A toxin receptor from *Manduca sexta*, expressed in mammalian and insect cell cultures, *Appl. Environ. Microbiol.*, Vol. 63, pp. 3419-3425, 1997.

T.P. Keeton et al., Effects of midgut-protein-preparative and ligand binding procedures on the toxin binding characteristics of BT-$R_1$, a common high-affinity receptor in *Manduca sexta* for Cry1A *Bacillus thuringiensis* toxins, *Appl. Environ. Microbiol.*, Vol 64, pp. 2158-2165, 1998.

C. Kintner, Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain, *Cell*, Vol. 69, pp. 225-236, 1992.

P.J. Knight et al., The receptor for *Bacillus thuringiensis* Cry1A(c) delta-endotoxin in the brush border membrane of the lepidopteran *Manduca sexta* is aminopeptidase *N. Mol. Microbiol.*, Vol. 11, pp. 429-436, 1994.

B.H. Knowles, Mechanism of action of *Bacillus thuringiensis* insecticidal δ-endotoxins, *Adv. Insect Physiol.*, Vol. 24, pp. 275-308, 1994.

K.A. Knudsen et al., A role for cadherins in cellular signaling and differentiation, *J. Cell Biochem. Suppl.*, Vol. 30-31, pp. 168-176, 1998.

J.S. Kwa et al., Toxicity and binding properties of the *Bacillus thuringiensis* delta-endotoxin Cry1C to cultured insect cells, *J. Invertebr. Pathol.*, Vol. 71, pp. 121-127, 1998.

U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, Vol. 227, pp. 680-685, 1970.

M.K. Lee et al., Inconsistencies in determining *Bacillus thuringiensis* toxin binding sites relationship by comparing competition assays with ligand blotting, *Biochem. Biophys. Res. Commun.*, Vol. 220, pp. 575-580, 1996.

M.K. Lee et al., Location of *Bombyx mori* receptor binding region of a *Bacillus thuringiensis* δ-endotoxin, *J. Biol. Chem.*, Vol. 267, pp. 3115-3121, 1992.

M.K. Lee et al., Resistance to *Bacillus thuringiensis* Cry1A delta-endotoxins in a laboratory-selected *Heliothis virescens* strain is related to receptor

alteration, *Appl. Environ. Microbiol.*, Vol. 61, pp. 3836-3842. 1995.

J. Li et al., Crystal structure of the insecticidal δ-endotoxin from *Bacillus thuringiensis* at 2.5 Å resolution, *Nature*, Vol. 353, pp. 815-821, 1991.

K. Luo et al., A 106 kDa from of aminopeptidase is a receptor for *Bacillus thuringiensis* Cry1C d-endotoxin in the brush border membrane of *Manduca sexta*, *Insect Biochem. Mole., Biol.*, Vol. 26, pp. 783-791, 1996.

K. Luo et al., Binding of *Bacillus thuringiensis* Cry1Ac toxin to aminopeptidase in susceptible and resistant Diamondback moths (*Plutella xylostella*), *Appl. Environ. Microbiol.*, Vol. 63, pp. 1024-1027, 1997.

A.C. Martinez-Ramirez et al., Ligand blot identification of a *Manduca sexta* midgut binding protein specific to three *Bacillus thuringiensis* Cry1A-type ICPs, *Biochem. Biophys Res. Commun.*, Vol. 201, No. 2, pp. 782-787, 1994.

J. Mengaud et al., E-cadherin is the receptor required for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells, *Cell*, Vol. 84; pp. 923-932, 1996.

E.G. Midboe, Characterization of the BT-R$_1$ gene and its expression in *Manduca sexta*, Ph.D. University of Wyoming, Laramie.

W.J. Moar et al., Development of *Bacillus thuringiensis* Cry1C resistance by *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae), *Appl. Environ. Microbiol.*, Vol. 61, pp. 2086-2092.

S.M. Mohamed, Unpublished data, 1999.

J. Muller-Cohn et al., *Spodoptera littoralis* (Lepidoptera: Noctuidae) resistance to Cry1C and cross-resistance to other *Bacillus thuringiensis* crystal toxins, *J. Econ. Entomol.*, Vol. 89, pp. 791-797, 1996.

Y. Nagamatsu et al., Cloning, sequencing, and expression of the *Bombyx mori* receptor for *Bacillus thuringiensis* insecticidal Cry1A(a) toxin, *Biosci. Biotechnol. Biochem.*, Vol. 62, pp. 727-734.

Y. Nagamatsu et al., Identification of *Bombyx mori* midgut receptor for *Bacillus thuringiensis* insecticidal Cry1A(a) toxin, *Biosci. Biotechnol. Biochem.*, Vol. 62, pp. 718-726, 1998.

P.H. O'Farrell, High resolution two-dimensional electrophoresis of proteins, *J. Biol. Chem.*, Vol. 250, pp. 4007-4021, 1975.

B. Oppert et al., Luminal proteinases from *Plodia interpunctella* and the hydrolysis of *Bacillus thuringiensis* Cry1A(c) protoxin, *Insect Biochem. Mol. Biol.*, Vol. 26, pp. 571-583, 1996.

J.J. Peluso et al., N-cadherin-mediated cell contact inhibits granulosa cell apoptosis in a progesterone-independent manner, *Endocrinology*, Vol. 137, pp. 1196-1203, 1996.

F.J. Perlak et al., Insect resistant cotton plants, *Biotechnology* (NY), Vol. 8, pp. 939-943, 1990.

C.T. Powell et al., Persistent membrane translocation of protein kinase C alpha during 12-0-

tetradecanoylphorbol-13-acetate-induced apoptosis of LNCaP human prostate cancer cells, *Cell Growth Differ.*, Vol. 7, pp. 419-428, 1996.

D.L. Rimm et al., Molecular cloning of human E-cadherin suggests a novel subdivision of the cadherin superfamily, *Biochem. Biophys. Res. Commun.*, Vol. 200, pp. 1754-1761, 1994.

J. Sambrook et al., Molecular Cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989.

E. Schnepf et al., *Bacillus thuringiensis* and its pesticidal crystal proteins, *Microbiol. Mol. Biol. Rev.*, Vol. 62, No. 3, pp. 775-806, 1998.

J.L. Schwartz et al., Single-site mutations in the conserved alternating-arginine region affect ionic channels formed by Cry1Aa, a *Bacillus thuringiensis* toxin, *Appl. Environ. Microbiol.*, Vol. 63, pp. 3978-3984, 1997.

T. Shimizu et al., Lamin B phosphorylation by protein kinase calpha and proteolysis during apoptosis in human leukemia HL60 cells, *J. Biol. Chem.*, Vol. 273, No. 15, pp. 8669-8674, 1998.

S. Strehl et al., Characterization of two novel protocadherins (PCDH8 and PCDH9) localized on human chromosome 13 and mouse chromosome 14, *Genomics*, Vol. 53, No. 1, pp. 81-89, 1998.

S.T. Suzuki, Protocadherins and diversity of the cadherin superfamily, *J. Cell, Sci.*, Vol. 109 (Pt. 11), pp. 2609-2611, 1996.

B.E. Tabashnik et al., Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylstella*, *PNAS* USA, Vol. 91, No. 10, pp. 4120-4124, 1994.

M. Takeichi et al., Cadherin-mediated cell-cell adhesion and neurogenesis, *Neurosci. Res. Suppl.*, Vol. 13, pp. S92-S96, 1990.

R.K. Vadlamudi et al., A specific binding protein from *Manduca sexta* for the insecticidal toxin of *Bacillus thuringiensis subsp. berliner.*, J. Biol. Chem., Vol. 268, No. 17, pp. 12334-12340, 1993.

R.K. Vadlamudi et al., Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis*, *J. Biol. Chem.*, Vol. 270, No. 10, pp. 5490-5494, 1995.

A.P. Valaitis et al., Interaction analyses of *Bacillus thuringiensis* Cry1A toxins with two aminopeptidases from gypsy moth midgut brush border membranes, *Insect Biochem. Mol. Biol.*, Vol. 27, pp. 529-539, 1997.

A.P. Valaitis et al., Brush border membrane aminopeptidase-N in the midgut of the gypsy moth serves as the receptor for the Cry1A(c) delta-endotoxin of *Bacillus thuringiensis*, *Insect Biochem. Mol. Biol.*, Vol. 25, No. 10, pp. 1143-1151, 1995.

T.F. Watson et al., Presented at the Beltwide Cotton Conf., Memphis.

M.E. Whalon et al., Selection of a Colorado potato beetle (Coleoptera: Chysomelidae) strain resistant to *Bacillus thuringiensis*, *J. Econ. Entomol.*, Vol. 86, pp. 226-233, 1993.

M.R. Williams, Presented at the Beltwide Cotton Conf., 1999.

G.K. Winkel et al., Activation of protein kinase C triggers premature compaction in the four-cell stage mouse embryo, *Dev. Biol.*, Vol. 138, pp. 1-15, 1990.

M.G. Wolfersberger, The toxicity of two *Bacillus thuringiensis* δ-endotoxins to gypsy moth larvae is inversely related to the affinity of binding sites on midgut brush border membrane for the toxins, *Experientia*, Vol. 46, pp. 475-477, 1990.

M. Wolfersberger et al., Preparation and partial characterization of amino acid transporting brush border membrane vesicles from the larval midgut of the cabbage butterfly (*Pieris brassicae*), *Comp. Biochem. Physiol.*, Vol. 86A, pp. 301-308, 1987.

K. Yaoi et al., Aminopeptidase N from *Bombyx mori* as a candidate for the receptor of *Bacillus thuringiensis* Cry1Aa toxin, *Eur. J. Biochem.*, Vol. 246, pp. 652-657, 1997.

While this invention has been described with reference to illustrative embodiments, this description is not intended to be construed in a limiting sense. Various modifications and combinations of illustrative embodiments, as well as other embodiments of the invention, will be apparent to persons skilled in the art upon reference to the description. It is therefore intended that the appended claims encompass such modifications and enhancements.

**WHAT IS CLAIMED IS:**

1. An isolated nucleic acid molecule, comprising a sequence encoding a BT toxin receptor of about 200 kD from the pink bollworm, *Pectinophora gossypiella*.

2. The isolated nucleic acid molecule of claim 1, encoding the BT toxin receptor sequence of SEQ ID NO: 2.

3. The isolated nucleic acid molecule of claim 1, comprising the sequence of SEQ ID NO: 1.

4. The isolated nucleic acid molecule of claim 1, wherein said isolated nucleic acid molecule is capable of hybridizing at high stringency to a probe of 400 contiguous nucleotides of SEQ ID NO: 1 over the entire length of said probe.

5. The isolated nucleic acid molecule of claim 1, wherein said BT toxin receptor is at least 85% homologous to the sequence of SEQ ID NO: 2.

6. The isolated nucleic acid molecule of claim 1, wherein said BT toxin receptor is at least 90% homologous to the sequence of SEQ ID NO: 2.

7. The isolated nucleic acid molecule of claim 1, wherein said BT toxin receptor is at least 95% homologous to the sequence of SEQ ID NO: 2.

8. The isolated nucleic acid molecule of claim 1, wherein said BT toxin receptor is at least 98% homologous to the sequence of SEQ ID NO: 2.

9. The isolated nucleic acid molecule of claim 1, wherein the sequence is codon optimized for expression in a prokaryotic system.

10. The isolated polynucleotide sequence of claim 1 wherein the sequence is codon optimized for expression in a eukaryotic system.

11. An expression vector, comprising the isolated nucleic acid molecule of claims 1-10.

12. A host cell, comprising the vector of claim 11.

13. The host cell of claim 12, which is used to assess the level of cytotoxicity and mode of action of toxins.

14. A cell, comprising a naturally occurring BT toxin receptor, which is used to assess the level of cytotoxicity and mode of action of toxins.

15. A transgenic organism, comprising the vector of claim 11.

16. An isolated nucleic acid molecule, comprising a sequence encoding a peptide selected from the group consisting of: amino acid (aa) 534-544, aa 291-304, aa 697-705, aa 622-632, aa 886-895, aa 791-803, aa 1055-1066, aa 1621-1642, aa 1321-1331, aa 1451-1461, aa 1516-1525, aa 1572-1582, aa 1677-1729, and aa 1269-1367 of SEQ ID NO: 2.

17. A purified protein, comprising a sequence that is at least 85% homologous to SEQ ID NO: 2:

18. The purified protein of claim 17, wherein the sequence is at least 90% homologous to SEQ ID NO: 2.

19. The purified protein of claim 17, wherein the sequence is at least 95% homologous to SEQ ID NO: 2.

20. The purified protein of claim 17, wherein the sequence is at least 98% homologous to SEQ ID NO: 2.

21. The purified protein of claim 17, wherein the sequence is SEQ ID NO: 2.

22. A purified peptide, comprising a sequence selected from the group consisting of; amino acid (aa) 534-544; aa 291-304; aa 697-705; aa 622-632; aa 886-895; aa 791-803; aa 1055-1066; aa 1621-1642; aa 1321-1331; aa 1451-1461; aa 1516-1525; aa 1572-1582; aa 1677-1729; and aa 1269-1367 of SEQ ID NO: 2.

23. The purified peptide of claim 22, that comprises aa 1269-1367 of SEQ ID NO: 2.

24. A purified peptide that comprises at least 17 contiguous amino acids (aa) from aa 1677-1729 of SEQ ID NO: 2.

25. An isolated nucleic acid molecule, comprising a first sequence that is capable of hybridizing at high stringency to a probe of a second sequence along said probes entire length, wherein said second sequence is nucleotides 3807-4101 of SEQ ID NO: 1.

26. An isolated nucleic acid molecule, comprising a sequence that encodes the peptide of claims 19-21.

27. *Bacillus thuringiensis* Cry toxin receptor antibody, that binds to an antigen present in the carboxyl tail of a BT-R2 protein as identified in SEQ ID NO: 2, and does not bind to silkworm or tobacco hornworm *Bacillus thuringiensis* Cry toxin receptors.

28. A *Bacillus thuringiensis* Cry toxin receptor antibody, that binds to an antigen present in a BT-R2 protein as identified in SEQ ID NO: 2, and also binds to silkworm and tobacco hornworm *Bacillus thuringiensis* Cry toxin receptors.

AACATTTACATACAGCCAGTGTAGATGACACATTGATTTAAAAAAAATAGTGCGAGTGCTTTGA
ATCTGTGATTTCAAATATCGAATCAAAAGGACTGCATTAGTGTTGTGGGAGTTAAAGTGTTTGT
GAGAATAGACCAACGACCATGCAAGATGGCGGGTGACGCCTGCATACTGGTGACGGTGCTTCTC
ACCTTCGCAACATCAGTTTTCGGGCAAGAAACAACATCGTCGAGATGTTACTACATGACTGACG
CTATTCCGAGGGAACCGAAACCGGATGATTTGCCTGACTTAGAATGGACTGGTGGATGGACCGA
CTGGCCTTTGATCCCGGCTGAGCCAAGAGACGACGTGTGCATAAACGGCTGGTACCCACAACTC
ACCAGCACTTCTCTCGGCACCATCATCATCCACATGGAAGAGGAGATCGAGGGAGATGTTGCTA
TCGCTAAACTTAACTATGATGGTTCTGGAACCCCAGAAATTGTCCAGCCGATGGTTATAGGATC
TTCTAACCTGCTAAGTCCAGAGATCCGGAATGAAAACGGGGCGTGGTACCTTTATATAACCAAT
AGGCAAGATTATGAAACACCAACAATGCGTCGGTATACATTCGACGTCCGAGTGCCAGACGAGA
CTCGTGCGGCACGAGTGAGTCTGTCCATCGAAAACATTGACGATAACGACCCTATCGTCAGGGT
GCTAGACGCTTGCCAAGTGCCGGAATTGGGGGAGCCTCGACTAACAGACTGCGTTTACCAAGTG
TCAGACGAAGATGGGAGGCTTAGTATCGAGCCCATGACATTCCGCCTCACATCAGACCGTGAAG
ACGTACAGATATTCTATGTGGAGCCAGCTCACATTACTGGTGATTGGTTCAACATGCAAATTAC
TATCGGTATCCTATCAGCGCTTAACTTCGAAAGCAACCCGCTGCACATCTTTCAAATCACTGCT
TTGGACTCCTGGCCCAACAACCATACGGTGACGGTGATGGTGCAAGTCCAGAATGTGGAACACC
GACCGCCGCGATGGATGGAAATCTTCGCAGTCCAGCAGTTTGACGAGATGACGGAGCAGCAATT
CCAGGTGCGCGCCATCGACGGAGACACTGGCATCGGGAAAGCTATACACTATACCCTCGAGACA
GATGAGGAAGAAGATTTGTTCTTCATCGAAACACTTCCGGGCGGCCATGACGGAGCCATCTTCA
GCACTGCCATGATTGATGTGGATAGGCTCCGGCGAGATGTCTTCAGACTGTCCCTGGTGGCATA
CAAGTACGACAATGTGTCCTTCGCCACCCCGACACCCGTCGTGATCATAGTCAATGACATCAAC
AACAAGAAACCCCAACCGCTGCAAGATGAGTACACAATCTCCATAATGGAAGAAACTCCACTGT
CGCTGAATTTTGCTGAACTTTTTGGTTTCTATGATGAAGATTTGATCTACGCACAATCCTTGGT
GGAAATACAAGGCGAGAACCCTCCAGGCGTAGAGCAAGCGTTTTATATTGCGCCCACCGCAGGC
TTCCAGAACCAGACATTCGCCATAGGGACTCAAGATCACCGAATGCTGGATTATGAGGATGTTC
CTTTCCAAAACATCAAGCTCAAGGTAATAGCAACGGACCGTGACAATACCAATTTTACTGGAGT
CGCGGAAGTCAACGTGAACCTGATTAATTGGAACGACGAGGAGCCGATCTTTGAGGAAGACCAG
CTCGTTGTCAAGTTCAAGGAGACTGTACCCAAGGACTATCACGTCGGCAGACTGAGGGCTCACG
ACCGGGACATAGGAGACAGCGTTGTGCATTCCATCTTGGGAAATGCGAATACATTTTTGAGAAT
CGACGAAGAAACTGGCGACATATACGTAGCTATTGATGACGCGTTCGATTATCACAGACAGAAT
GAATTTAACATACAAGTTCGCGCTCAGGACACCATGTCGGAGCCAGAGTCCAGGCATACAGCGG
CTGCTCAGCTGGTCATAGAACTCGAGGACGTCAACAACACACCTCCTACTCTGAGGCTGCCTCG
CGTAAGTCCGTCTGTAGAAGAGAATGTGCCAGAGGGCTTTGAAATCAACCGGGAGATAACCGCC
ACGGACCCTGACACCACAGCATACCTGCAGTTTGAAATAGATTGGGACACATCCTTTGCCACTA
AACAGGGGCGTGATACCAATCCAATAGAGTTCCACGGATGCGTGGATATAGAAACCATCTTCCC
AAACCCAGCCGACACCAGAGAGGCTGTGGGGCGAGTGGTAGCGAAGGGGATCCGCCATAACGTG
ACCATCCATTTTGAAGAGTTTGAATTTCTCTACCTCACAGTGAGAGTTCGGGACTTGCACACAG
ATGACGGACGAGATTATGATGAATCTACCTTCACGGTAATAATAATAGATATGAACGACAACTG
GCCTATCTGGGCGTCTGGTTTCCTGAACCAGACCTTCAGTATTCGGGAGCGATCATCTACCGGC
GTCGTCATCGGGTCCGTACTCGCTACAGACATTGATGGCCCACTTTACAACCAAGTCCGGTACA
CCATTATCCCCCAGGAAGATACTCCTGAAGGTCTAGTCCAGATACATTTCGTTACGGGTCAAAT
TACAGTTGATGAGAATGGTGCAATCGACGCTGATATTCCACCTCGTTGGCACCTCAACTACACG
GTTATAGCCAGCGACAAATGTTCTGAAGAAAATGAAGAGAACTGTCCCCCGGATCCAGTGTTCT
GGGATACTCTGCGCGACAATGTAATTAACATCGTGGACATAAACAACAAGGTCCCGGCAGCAGA
CCTCAGTCGATTCAACGAAACGGTGTACATTTATGAAAATGCACCCGATTTCACGAACGTGGTC

Figure 1A

AAGATATACTCCATCGACGAAGACAGAGACGAAATATATCACACGGTGCGGTACCAGATCAATT
ATGCTGTGAACCAACGGCTGCGAGACTTCTTCGCCATAGACCTGGATTCAGGCCAGGTGTACGT
GGAGAACACCAACAATGAGCTCCTGGATCGGGACAGAGGCGAAGACCAACACAGGATATTCATT
AACCTCATTGACAACTTTTATAGCGAAGGAGATGGAAATAGAAATGTAAACACTACAGAGGTGC
TGGTGATACTATTAGATGAGAATGACAACGCTCCTGAATTGCCGACTCCAGAAGAGCTGAGTTG
GAGCATTTCCGAGGATTTACAAGAGGGTATAACACTCGATGGCGAAAGCGATGTGATATACGCA
CCGGATATAGACAAAGAGGACACGCCAAACTCTCACGTTGGCTACGCAATCCTGGCCATGACAG
TCACCAATAGAGACCTGGACACTGTTCCGAGACTTCTCAACATGCTGTCGCCTAACAACGTAAC
CGGATTCCTCCAGACAGCAATGCCTTTGAGAGGATATTGGGGGACTTACGATATAAGTGTACTG
GCGTTCGACCACGGTATTCCTCAGCAGATATCTCATGAGGTGTATGAATTGGAAATTCGACCTT
ACAATTACAATCCTCCTCAGTTCGTTTTTCCTGAATCCGGGACGATTCTACGACTGGCTTTGGA
ACGCGCAGTGGTAAATAATGTTTTGTCACTTGTAAACGGTGACCCGTTAGACAGGATACAAGCA
ATTGACGACGATGGTCTTGATGCTGGCGTGGTGACTTTCGATATTGTTGGAGATGCTGATGCGT
CAAACTACTTCAGAGTAAATAATGATGGCGACAGCTTTGGAACCTTGTTGCTGACACAGGCGCT
TCCTGAGGAAGGCAAGGAATTTGAGGTTACCATCCGGGCTACAGACGGCGGAACAGAACCTCGA
TCATATTCAACAGACTCCACTATAACAGTCCTCTTCGTTCCGACTTTGGGTGATCCGATCTTTC
AAGATAACACTTACTCAGTAGCATTCTTTGAAAAAGAGGTTGGCTTGACTGAGAGGTTCTCGCT
CCCACATGCAGAGGACCCTAAGAACAAACTCTGCACTGACGACTGTCACGATATTTACTACAGG
ATCTTTGGTGGTGTGGATTACGAGCCATTTGACCTGGACCCGGTGACGAACGTGATCTTCCTGA
AATCAGAACTAGACCGGGAGACCACTGCTACGCATGTGGTGCAAGTGGCAGCCAGTAATTCGCC
CACAGGAGGCGGAATACCACTCCCTGGGTCTCTTCTCACCGTCACTGTCACTGTACGAGAAGCG
GATCCACGGCCTGTGTTCGAGCAGCGTCTGTACACGGCTGGCATTTCCACTTCCGATAACATCA
ACAGGGAACTACTCACCGTTCGTGCAACTCATTCCGAAAACGCACAATTGACATATACCATCGA
AGACGGTTCTATGGCGGTGGACTCCACTCTGGAAGCCGTCAAGGACTCGGCGTTCCATCTGAAC
GCGCAGACCGGCGTCCTCATACTGAGGATACAACCTACTGCCAGCATGCAGGGCATGTTTGAGT
TCAACGTCATCGCTACTGACCCAGATGAGAAGACAGATACGGCAGAGGTGAAAGTCTACCTCAT
TTCATCCCAAAATAGGGTGTCCTTCATATTCCTGAACGATGTGGAGACGGTTGAGAGTAACAGA
GACTTTATCGCAGAAACGTTCAGCGTTGGCTTCAACATGACCTGCAATATAGATCAGGTGCTGC
CGGGCACCAACGACGCCGGGGTGATTCAGGAGGCCATGGCGGAAGTCCATGCTCACTTCATACA
GGATAACATCCCTGTGAGCGCCGACAGTATTGAAGAGCTTCGCAGTGACACTCAGCTGCTGCGC
TCCGTCCAAGGTGTGTTGAACCAACGGCTGTTGGTCCTGAACGACCTGGTGACGGGGGTCAGCC
CTGATCTCGGCACTGCCGGCGTGCAGATCACCATCTATGTGCTAGCCGGGTTGTCAGCCATCCT
TGCCTTCCTGTGCCTTATTCTGCTCATCACATTCATCGTGAGGACCCGAGCTCTGAACCGCCGT
TTGGAAGCACTGTCGATGACGAAATACGGCTCGGTGGATTCGGGGCTGAACCGAGTGGGGATAG
CGGCCCCAGGAACCAACAAACACGCCATCGAAGGCTCCAACCCCATCTGGAACGAGCAGATCAA
GGCCCCGGACTTCGATGCCATCAGTGACACATCTGACGACTCTGATCTAATCGGCATCGAGGAT
AGCCTGCAGGGAGACTTAGAAGAGAAAAGGGCAGACAAAGCAGTAGATGCCTTGGTGAAAAAGC
TGAAGAAGAACGATGGAGCCATGGGGGAATACGAATTCAAGGCCTCTCGAGCCTCTAGAACTAT
CGTGAGTCGTATTACGTATATCCAGACATGATGAGATACATTGATGAGTTTGGACAAACCGCAA
CTAGAATGCAGTGAAAAAAATGCTTTATTTGTTGAAATTTGTGATGCTATTGCTTTATTTGGAA
CCATTATAAGCTGCAATAAACAAGTTAACATCATCAATTGCATTCATTTTATGTTTCAGGTTCA
GGGGGAGGTGTGGGAGGCTATCC

Figure 1B

SIG 3/9

1 MAGDACILVT VLLTFATSVF GQETTSSRCY YMTDAIPREP KPDDLPDLEW

CR1 →

51 TGGWTDWPLI PAEPRDDVCI NGWYPQLTST SLGTIIIHME EEIEGDVAIA

101 KLNYDGSGTP EIVQPMVIGS SNLLSPEIRN ENGAWYLYIT NRQDYETPTM

CR2 →

151 RRYTFDVRVP DETRAARVSL SIENIDDNDP IVRVLDACQV PELGEPRLTD

201 CVYQVSDEDG RLSIEPMTFR LTSDREDVQI FYVEPAHITG DWFNMQITIG

CR3 →

251 ILSALNFESN PLHIFQITAL DSWPNNÑHTVT VMVQVQNVEH RPPRWMEIFA

301 VQQFDEMTEQ QFQVRAIDGD TGIGKAIHYT LETDEEEDLF FIETLPGGHD

351 GAIFSTAMID VDRLRRDVFR LSLVAYKYDÑ VSFATPTPVV IIVNDINNKK

CR4 →

401 PQPLQDEYTI SIMEETPLSL NFAELFGFYD EDLIYAQSLV EIQGENPPGV

451 EQAFYIAPTA GFQÑQTFAIG TQDHRMLDYE DVPFQNIKLK VIATDRDNTÑ

CR5 →

501 FTGVAEVNVN LINWNDEEPI FEEDQLVVKF KETVPKDYHV GRLRAHDRDI

551 GDSVVHSILG NANTFLRIDE ETGDIYVAID DAFDYHRQNE FNIQVRAQDT

CR6 →

601 MSEPESRHTA AAQLVIELED VNNTPPTLRL PRVSPSVEEN VPEGFEINRE

651 ITATDPDTTA YLQFEIDWDT SFATKQGRDT NPIEFHGCVD IETIFPNPAD

701 TREAVGRVVA KGIRHÑVTIH FEEFEFLYLT VRVRDLHTDD GRDYDESTFT

CR7 →

751 VIIIDMNDNW PIWASGFLÑQ TFSIRERSST GVVIGSVLAT DIDGPLYNQV

801 RYTIIPQEDT PEGLVQIHFV TGQITVDENG AIDADIPPRW HLÑYTVIASD

CR8 →

851 KCSEENEENC PPDPVFWDTL RDNVINIVDI NNKVPAADLS RFÑETVYIYE

901 NAPDFTNVVK IYSIDEDRDE IYHTVRYQIN YAVNQRLRDF FAIDLDSGQV

951 YVENTNNELL DRDRGEDQHR IFINLIDNFY SEGDGNRNVÑ TTEVLVILLD

CR9 →

1001 ENDNAPELPT PEELSWSISE DLQEGITLDG ESDVIYAPDI DKEDTPNSHV

1051 GYAILAMTVT NRDLDTVPRL LNMLSPNÑVT GFLQTAMPLR GYWGTYDISV

1101 LAFDHGIPQQ ISHEVYELEI RPYNYNPPQF VFPESGTILR LALERAVVNN

Figure 2A

CR10 →

1151 VLSLVNGDPL DRIQAIDDDG LDAGVVTFDI VGDADASNYF RVNNDGDSFG

1201 TLLLTQALPE EGKEFEVTIR ATDGGTEPRS YSTDSTITVL FVPTLGDPIF

CR11→ MBF

1251 QDNTYSVAFF EKEVGLTERF SLPHAEDPKN KLCTDDCHDI YYRIFGGVDY

1301 EPFDLDPVTN VIFLKSELDR ETTATHVVQV AASNSPTGGG IPLPGSLLTV

CR12→

1351 TVTVREADPR PVFEQRLYTA GISTSDNINR ELLTVRATHS ENAQLTYTIE

1401 DGSMAVDSTL EAVKDSAFHL NAQTGVLILR IQPTASMQGM FEFNVIATDP

→ MPD

1451 DEKTDTAEVK VYLISSQNRV SFIFLNDVET VESNRDFIAE TFSVGFNMTC

LZ

1501 NIDQVLPGTN DAGVIQEAMA EVHAHFIQDN IPVSADSIEE LRSDTQLLRS

1551 VQGVLNQRLL VLNDLVTGVS PDLGTAGVQI TIYVLAGLSA ILAFLCLILL

→ CYT

1601 ITFIVRTRAL NRRLEALSMT KYGSVDSGLN RVGIAAPGTN KHAIEGSNPI

1651 WNEQIKAPDF DAISDTSDDS DLIGIEDSLQ GDLEEKRADK AVDALVKKLK

1701 KNDGAMGEYE FKASRASRTI VSRITYIQT.

Figure 2B

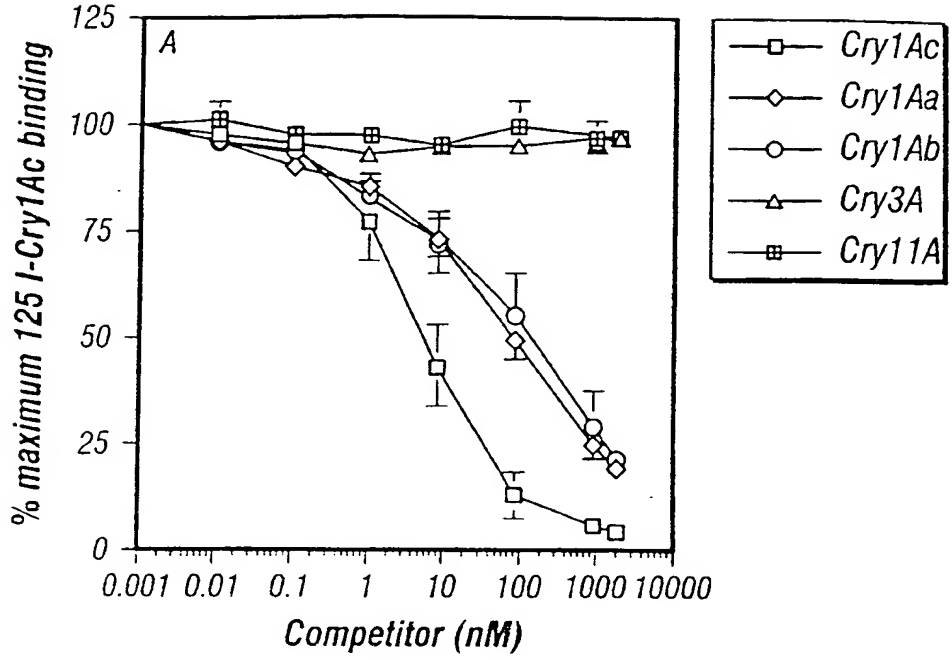

A
125
100
75
50
25
0
% maximum 125 I-Cry1Ac binding
0.001 0.01 0.1 1 10 100 1000 10000
Competitor (nM)
Cry1Ac
Cry1Aa
Cry1Ab
Cry3A
Cry11A

FIG. 3A

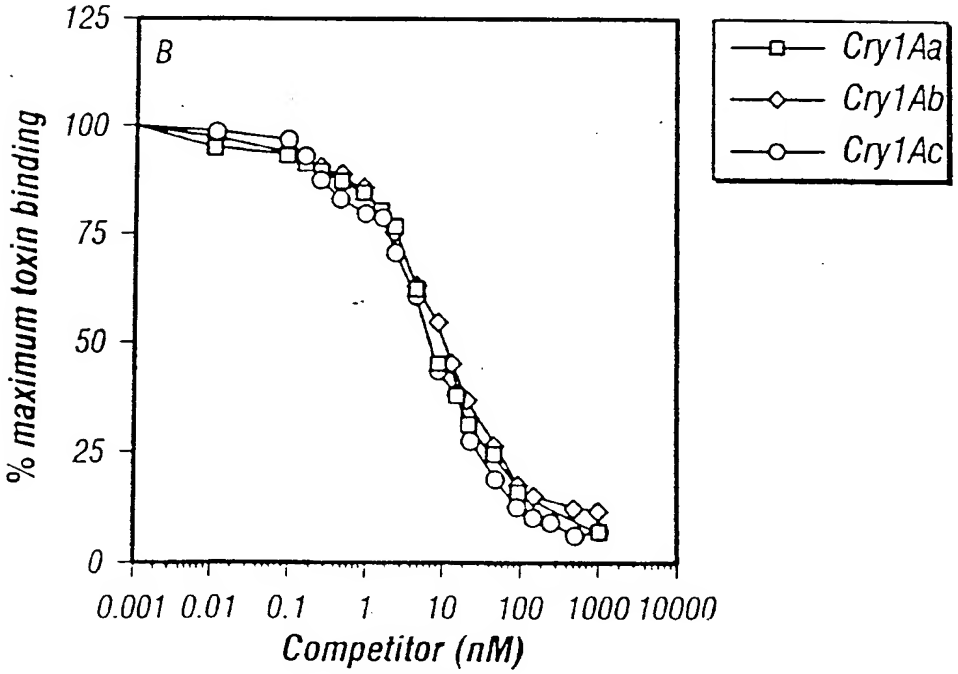

B
125
100
75
50
25
0
% maximum toxin binding
0.001 0.01 0.1 1 10 100 1000 10000
Competitor (nM)
Cry1Aa
Cry1Ab
Cry1Ac

FIG. 3B

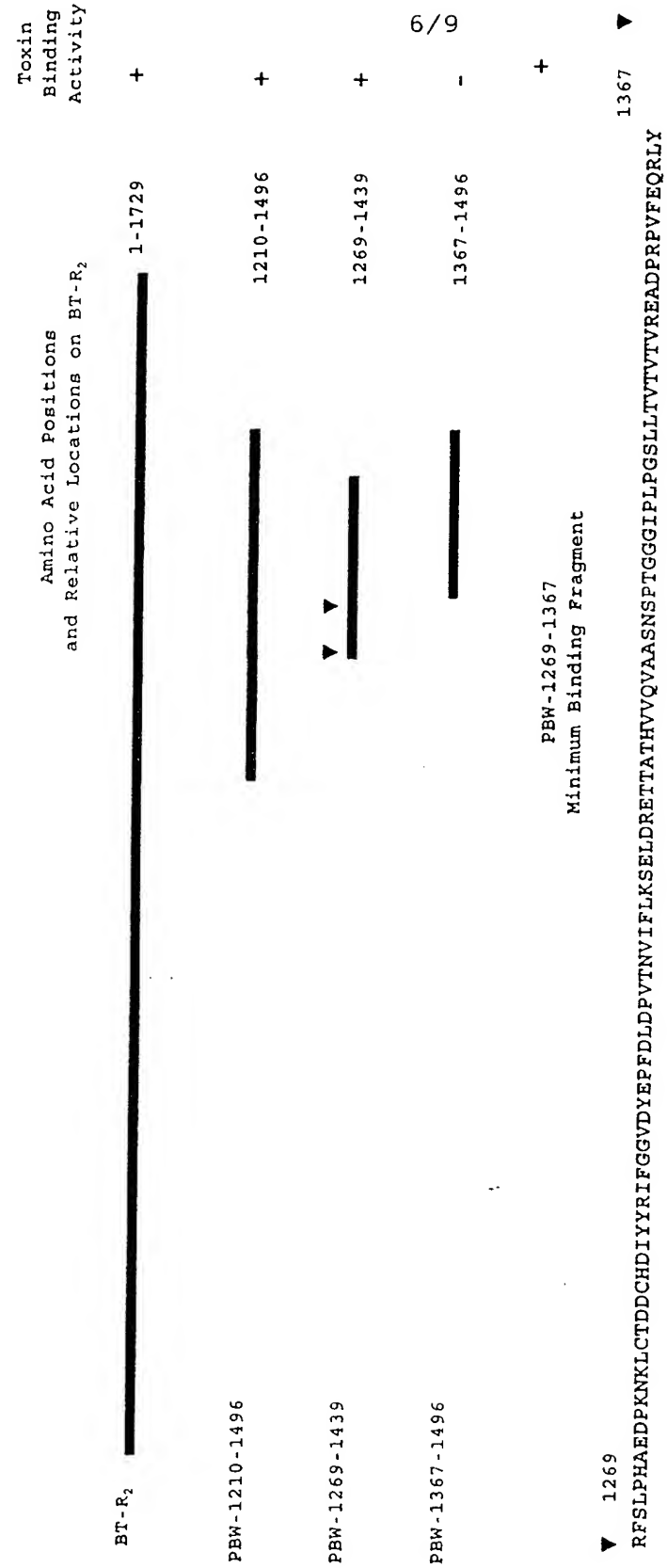
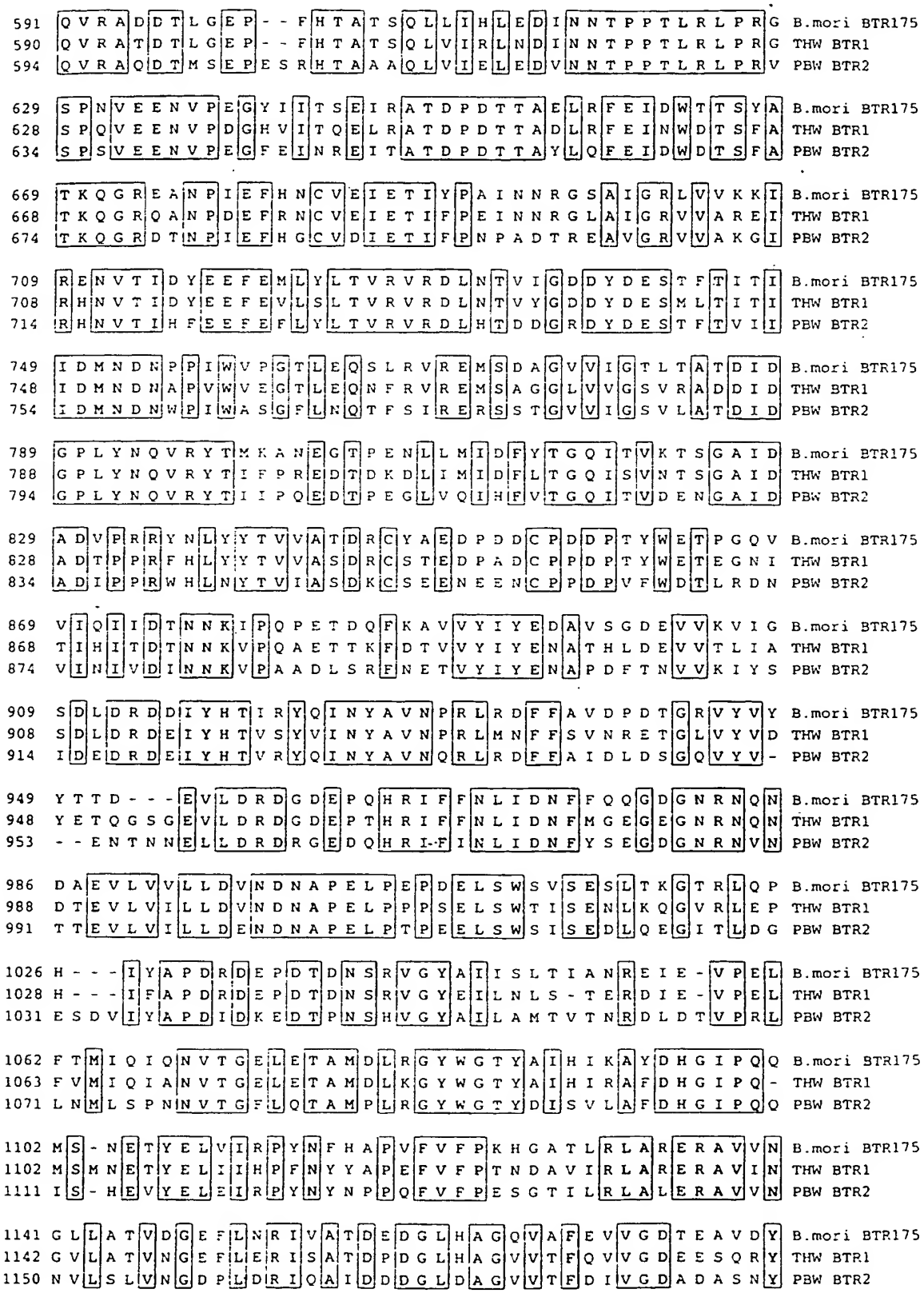

Toxin Binding Activity
Amino Acid Positions and Relative Locations on BT-R2
BT-R2
1-1729
+
PBW-1210-1496
1210-1496
+
PBW-1269-1439
1269-1439
+
PBW-1367-1496
1367-1496
-
PBW-1269-1367 Minimum Binding Fragment
+
1269
1367
RFSLPHAEDPKNKLCTDDCHDIYYRIFGGVDYEPFDLDPVTNVIFLKSELDRETTATHVVQVAASNSPTGGGIPLPGSLLTVTVTVREADPRPVFEQRLY

Figure 4

# Figure 5A


1 MGVDVRILATLLIY-AETVLAQE----RCGFMV-AIPRP B.mori BTR175
1 MAVDVRI-AAFLLVFIAPAVLAQE----RCGYMT-AIPRL THW BTR1
1 MAGDACILVTVLLTF-ATSVFGQETTSSRCYYMTDAIPRE PBW BTR2
35 PRPD-LPELDFEGQTWSQRPLIPAADREDVCMDG-YHAMT B.mori BTR175
35 PRPDNLPVLNFEGQTWSQRPLLPAPERDDLCMDA-YHVIT THW BTR1
40 PKPDDLPDLEWTG-GWTDWPLIPAEPRDDVCINGWYPQLT PBW BTR2
73 PT-YGTQIIYMEEEIEGEVPIAKLNYRGPNVPYIEPAFLS B.mori BTR175
74 AN-LGTQVIYMDEEIEDEITIAILNYNGPSTPFIELPFLS THW BTR1
79 STSLGTIIIHMEEEIEGDVAIAKLNYDGSGTPEIVQPMVI PBW BTR2
112 GSFNLLVPVIRRIPDSNGEWHLIITQRQDYETPGMQQYVF B.mori BTR175
113 GSYNLLMPVIRRV--DNGEWHLIITQRQHYELPGMQQYMF THW BTR1
119 GSSNLLSPEIR---NENGAWYLYITNRQDYETPTMRRYTF PBW BTR2
152 NIRIDGETLVAGVSLLIVNIDDNAPIIQALEPCQVDELGE B.mori BTR175
151 NVRVDGQSLVAGVSLAIVNIDDNAPIIQNFEPCRVPELGE THW BTR1
156 DVRVPDETRAARVSLSIENIDDNDPIVRVLDACQVPELGE PBW BTR2
192 ARLTECVYVVTDADGRISTQFMQFRIDSDRGDDKIFYIQG B.mori BTR175
191 PGLTECTYQVSDADGRISTEFMTFRIDSVRGDEETFYIER THW BTR1
196 PRLTDCVYQVSDEDGRLSIEPMTFRLTSDREDVQIFYVEP PBW BTR2
232 ANIPGEWIRMTMTVGINEPLNFETNPLHIFSVTALDSLPN B.mori BTR175
231 TNIPNQWMWLNMTIGVNTSLNFVTSPLHIFSVTALDSLPN THW BTR1
236 AHITGDWFNMQITIGILSALNFESNPLHIFQITALDSWPN PBW BTR2
272 THTVTLMVQVENVEHRPPRWVEIFAVQQFDEKTAQSFPVR B.mori BTR175
271 THTVTMMVQVANVNSRPPRWLEIFAVQQFEEKSYQNFTVR THW BTR1
276 NHTVTVMVQVQNVEHRPPRWMEIFAVQQFDEMTEQQFQVR PBW BTR2
312 AIDGDTGINKPIHYRLETAEEDTFFHIRTIEGGRSGAILY B.mori BTR175
311 AIDGDTEINMPINYRLITNEEDTFFSIEALPGGKSGAVFL THW BTR1
316 AIDGDTGIGKAIHYTLETDEEEDLFFIETLPGGHDGAIFS PBW BTR2
352 VDPIDRDTLQREVFQLSIIAYKYDNESSATAANVVIIVND B.mori BTR175
351 VSPIDRDTLQREVFPLTIVAYKYDEEAFSTSTNVVIIVTD THW BTR1
356 TAMIDVDRLRRDVFRLSLVAYKYDNVSFATPTPVVIIVND PBW BTR2
392 INDQRPEPLFKEYRLNIMEETALTLNFDQEFGFHDRDLGQ B.mori BTR175
391 INDQRPEPIHKEYRLAIMEETPLTLNFDKEFGFHDKDLGQ THW BTR1
396 INNKKPQPLQDEYTISIMEETPLSLNFAELFGFYDEDL-I PBW BTR2
432 NAQYTVRLESDYPADAAKAFYIAPEVGYQRQTFIMGTANH B.mori BTR175
431 NAQYTVRLESVDPPGAAEAFYIAPEVGYQRQTFIMGTLNH THW BTR1
435 YAQSLVEIQGENPPGVEQAFYIAPTAGFQNQTFAIGTQDH PBW BTR2
472 KMLDYE-VPEFQRIRLRVIATDMDNEEHVGVAYVYINLIN B.mori BTR175
471 SMLDYE-VPEFQSITIRVVATDNNDTRHVGVALVHIDLIN THW BTR1
475 RMLDYEDVP-FQNIKLKVIATDRDNTNFTGVAEVNVNLIN PBW BTR2
511 WNDEEPIFEHSVQNVSFKETEGKGFFVANVRAHDRDIDDR B.mori BTR175
510 WNDEQPIFEHAVQTVTFDETEGEGFFVAKAVAHDRDIGDV THW BTR1
514 WNDEEPIFEEDQLVVKFKETVPKDYHVGRLRAHDRDIGDS PBW BTR2
551 VEHTLMGNANNYLSIDKDTGDIHVTQDDFFDYHRQSELFV B.mori BTR175
550 VEHTLLGNAVNFLTIDKLTGDIRVSANDSFNYHRESELFV THW BTR1
554 VVHSILGNANTFLRIDEETGDIYVAIDDAFDYHRQNEFNI PBW BTR2

# Figure 5B


591 QVRADDTLGEP--FHTATSQLLIHLEDINNTPPTLRLPRG B.mori BTR175
590 QVRATDTLGEP--FHTATSQLVIRLNDINNTPPTLRLPRG THW BTR1
594 QVRAQDTMSEPESRHTAAAQLVIELEDVNNTPPTLRLPRV PBW BTR2
629 SPNVEENVPEGYIITSEIRATDPDTTAELRFEIDWTTSYA B.mori BTR175
628 SPQVEENVPDGHVITQELRATDPDTTADLRFEINWDTSFA THW BTR1
634 SPSVEENVPEGFEINRETATDPDTTAYLQFEIDWDTSFA PBW BTR2
669 TKQGREANPIEFHNCVEIETIYPAINNRGSAIGRLVVKKI B.mori BTR175
668 TKQGRQANPDEFRNCVEIETIFPEINNRGLAIGRVVARET THW BTR1
674 TKQGRDTNPIEFHGCVDIETIFPNPADTREAVGRVVAKGI PBW BTR2
709 RENVTIDYEEFEMLYLTVRVRDLNTVIGDDYDESTFTITI B.mori BTR175
708 RHNVTIDYEEFEVLSLTVRVRDLNTVYGDDYDESMLTITI THW BTR1
714 RHNVTIHFEEFEFLYLTVRVRDLHTDDGRDYDESTFTVII PBW BTR2
749 IDMNDNPPIWVPGTLEQSLRVREMSDAGVVIGTLTATDID B.mori BTR175
748 IDMNDNAPVWVEGTLEQNFRVREMSAGGLVVGSVRADDID THW BTR1
754 IDMNDNWPIWASGFLNQTFSIRERSSTGVVIGSVLATDID PBW BTR2
789 GPLYNQVRYTMKANEGTPENLLMIDFYTGQITVKTSGAID B.mori BTR175
788 GPLYNQVRYTIFPREDTDKDLIMIDFLTGQISVNTSGAID THW BTR1
794 GPLYNQVRYTIIPQEDTPEGLVQIHFVTGQITVDENGAID PBW BTR2
829 ADVPRRYNLYYTVVATDRCYAEDPDDCPDDPTYWETPGQV B.mori BTR175
828 ADTPPRFHLYYTVVASDRCSTEDPADCPPDPTYWETEGNI THW BTR1
834 ADIPPRWHLNYTVIASDKCSEENEENCPPDPVFWDTLRDN PBW BTR2
869 VIQIIDTNNKIPQPETDQFKAVVYIYEDAVSGDEVVKVIG B.mori BTR175
868 TIHITDTNNKVPQAETTKFDTVVYIYENATHLDEVVTLIA THW BTR1
874 VINIVDINNKVPAADLSRFNETVYIYENAPDFTNVVKIYS PBW BTR2
909 SDLDRDDIYHTIRYQINYAVNPRLRDFFAVDPDTGRVYVY B.mori BTR175
908 SDLDRDEIYHTVSYVINYAVNPRLMNFFSVNRETGLVYVD THW BTR1
914 IDEDRDEIYHTVRYQINYAVNQRLRDFFAIDLDSGQVYV- PBW BTR2
949 YTTD---EVLDRDGDEPQHRIFFNLIDNFFQQGDGNRNQN B.mori BTR175
948 YETQGSGEVLDRDGDEPTHRIFFNLIDNFMGEGEGNRNQN THW BTR1
953 --ENTNNELLDRDRGEDQHRI-FINLIDNFYSEGDGNRNVN PBW BTR2
986 DAEVLVVLLDVNDNAPELPEPDELSWSVSESLTKGTRLQP B.mori BTR175
988 DTEVLVILLDVNDNAPELPPPSELSWTISENLKQGVRLEP THW BTR1
991 TTEVLVILLDENDNAPELPTPEELSWSISEDLQEGITLDG PBW BTR2
1026 H---IYAPDRDEPDTDNSRVGYAIISLTIANREIE-VPEL B.mori BTR175
1028 H---IFAPDRDEPDTDNSRVGYEILNLS-TERDIE-VPEL THW BTR1
1031 ESDVIYAPDIDKEDTPNSHVGYAILAMTVTNRDLDTVPRL PBW BTR2
1062 FTMIQIQNVTGELETAMDLRGYWGTYAIHIKAYDHGIPQQ B.mori BTR175
1063 FVMIQIANVTGELETAMDLKGYWGTYAIHIRAFDHGIPQ- THW BTR1
1071 LNMLSPNNVTGFLQTAMPLRGYWGTYDISVLAFDHGIPQQ PBW BTR2
1102 MS-NETYELVIRPYNFHAPVFVFPKHGATLRLARERAVVN B.mori BTR175
1102 MSMNETYELIIHPFNYYAPEFVFPTNDAVIRLARERAVIN THW BTR1
1111 IS-HEVYELEIRPYNYNPPQFVFPESGTILRLALERAVVN PBW BTR2
1141 GLLATVDGEFLNRIVATDEDGLHAGQVAFEVVGDTEAVDY B.mori BTR175
1142 GVLATVNGEFLERISATDPDGLHAGVVTFQVVGDEESQRY THW BTR1
1150 NVLSLVNGDPLDRIQAIDDDGLDAGVVTFDIVGDADASNY PBW BTR2

# Figure 5C


1181 F H I V N D G E N S G T L M L K Q L F P E D I R E F E V T I R A T D G G T E P R B.mori BTR175
1182 F Q V V N D G E N L G S L R L L Q A V P E E I R E F R I T I R A T D Q G T D P G THW BTR1
1190 F R V N N D G D S F G T L L L T Q A L P E E G K E F E V T I R A T D G G T E P R PBW BTR2
1221 P L S T D C T F S V V F V P I Q G E P I F P T S T H T V A F I E K E A G L L E R B.mori BTR175
1222 P L S T D M T F R V V F V P T Q G E P R F A S S E H A V A F I E K S A G M E E S THW BTR1
1230 S Y S T D S T I T V L F V P T L G D P I F Q D N T Y S V A F F E K E V G L T E R PBW BTR2
1261 H E L P R A E D R K N H L C S D D C H N I Y Y R I I D G N N D G H F G L D E T T B.mori BTR175
1262 H Q L P L A Q D I K N H L C E D D C H S I Y Y R I I D G N S E G H F G L D P V R THW BTR1
1270 F S L P H A E D P K N K L C T D D C H D I Y Y R I F G G V D Y E P F D L D P V T PBW BTR2
1301 N V L F L V K E L D R S V S E T Y T L T I A A S N S P T G G - I A L T S T I - T B.mori BTR175
1302 N R L F L K K E L I R E Q S A S H T L Q V A A S N S P D G G - I P L P A S I L T THW BTR1
1310 N V I F L K S E L D R E T T A T H V V Q V A A S N S P T G G G I P L P G S L L T PBW BTR2
1339 I T V N V R E A D P Q P Y F V R D L Y T A G I S T S D S I N R E L L I L Q A T H B.mori BTR175
1341 V T V T V R E A D P R P V F V R E L Y T A G I S T A D S I G R E L L R L H A T Q THW BTR1
1350 V T V T V R E A D P R P V F E Q R L Y T A G I S T S D N I N R E L L T V R A T H PBW BTR2
1379 S E N A P I I Y T I D W S T M V T D P T L A S V R E T A F I L N P H T G V L T L B.mori BTR175
1381 S E G S A I T Y A I D Y D T M V V D P S L E A V R Q S A F V L N A Q T G V L T L THW BTR1
1390 S E N A Q L T Y T I E D G S M A V D S T L E A V K D S A F H L N A Q T G V L I L PBW BTR2
1419 N I Q P T A S M H G M F E F Q V V A T D P A G Y S D R A N V K I Y L I S T R N R B.mori BTR175
1421 N I Q P T A T M H G L F K F E V T A T D T A G A Q D R T D V T V Y V V S S Q N R THW BTR1
1430 R I Q P T A S M Q G M F E F N V I A T D P D E K T D T A E V K V Y L I S S Q N R PBW BTR2
1459 V F F L F V N T L E Q V E Q N T D F I A Q T F S A G F E M T C N I D Q V V P A T B.mori BTR175
1461 V Y F V F V N T L Q Q V E D N R D F I A D T F S A G F N M T C N I D Q V V P A N THW BTR1
1470 V S F I F L N D V E T V E S N R D F I A E T F S V G F N M T C N I D Q V L P G T PBW BTR2
1499 D A - S G V I M N G I T E V R G H F I R D N V P V P A D E I E T L R G D M V L L B.mori BTR175
1501 D P V T G V A L E H S T Q M R G H F I R D N V P V L A D E I E Q I R S D L V L L THW BTR1
1510 N D - A G V I Q E A M A E V H A H F I Q D N I P V S A D S I E E L R S D T Q L L PBW BTR2
1538 T A I Q S T L A T R L L V L R D L F T D T S P A - P D A G S A A V L Y A L A V L B.mori BTR175
1541 S S I Q T T L A A R S L V L Q D L L T N S S P D - S A P D S S L T V Y V L A S L THW BTR1
1549 R S V Q G V L N Q R L L V L N D L V T G V S P D L G T A G V Q I T I Y V L A G L PBW BTR2
1577 S A L L A A L C L L L L V I F I I R T K K L N R R L E A L T V K K Y G S V D S G B.mori BTR175
1580 S A V L G F M C L V L L L T F I I R T R A L N R R L E A L S M T K Y G S L D S G THW BTR1
1589 S A I L A F L C L I L L I T F I V R T R A L N R R L E A L S M T K Y G S V D S G PBW BTR2
1617 L N R V G I A A P G T N K H A V E G S N P I W N E T I K A P D F D S M S D A S N B.mori BTR175
1620 L N R A G I A A P G T N K H T V E G S N P I F N E A I K T P D L D A I S E G S N THW BTR1
1629 L N R V G I A A P G T N K H A I E G S N P I W N E Q I K A P D F D A I S D T S D PBW BTR2
1657 D S D L I G I E D L P H F G E N N Y F P R D V D E F K T D K - P E D I V A T H N B.mori BTR175
1660 D S D L I G I E D L P H F G - N V F M D P E V N E - K A N G Y P E - - V A N H N THW BTR1
1669 D S D L I G I E D S - - - - - - - - L Q G D L E E K R A D K A V D A L V K K L K PBW BTR2
1696 N N - - - - - F G F K S T P F S P E F A N - - Q F Q K B.mori BTR175
1696 N N - - - - - F A F N P T P F S P E F V N G - Q F R K I THW BTR1
1701 K N D G A M G E Y E F K A S R A S R T I V S R I T Y I Q T PBW BTR2

AACATTTACATACAGCCAGTGTAGATGACACATTGATTTAAAAAAAATAGTGCGAGTGCTTTGA
ATCTGTGATTTCAAATATCGAATCAAAAGGACTGCATTAGTGTTGTGGGAGTTAAAGTGTTTGT
GAGAATAGACCAACGACCATGCAAGATGGCGGGTGACGCCTGCATACTGGTGACGGTGCTTCTC
ACCTTCGCAACATCAGTTTTCGGGCAAGAAACAACATCGTCGAGATGTTACTACATGACTGACG
CTATTCCGAGGGAACCGAAACCGGATGATTTGCCTGACTTAGAATGGACTGGTGGATGGACCGA
CTGGCCTTTGATCCCGGCTGAGCCAAGAGACGACGTGTGCATAAACGGCTGGTACCCACAACTC
ACCAGCACTTCTCTCGGCACCATCATCATCCACATGGAAGAGGAGATCGAGGGAGATGTTGCTA
TCGCTAAACTTAACTATGATGGTTCTGGAACCCCAGAAATTGTCCAGCCGATGGTTATAGGATC
TTCTAACCTGCTAAGTCCAGAGATCCGGAATGAAAACGGGGCGTGGTACCTTTATATAACCAAT
AGGCAAGATTATGAAACACCAACAATGCGTCGGTATACATTCGACGTCCGAGTGCCAGACGAGA
CTCGTGCGGCACGAGTGAGTCTGTCCATCGAAAACATTGACGATAACGACCCTATCGTCAGGGT
GCTAGACGCTTGCCAAGTGCCGGAATTGGGGCAGCCTCGACTAACAGACTGCGTTTACCAAGTG
TCAGACGAAGATGGGAGGCTTAGTATCGAGCCCATGACATTCCGCCTCACATCAGACCGTGAAG
ACGTACAGATATTCTATGTGGAGCCAGCTCACATTACTGGTGATTGGTTCAACATGCAAATTAC
TATCGGTATCCTATCAGCGCTTAACTTCGAAAGCAACCCGCTGCACATCTTTCAAATCACTGCT
TTGGACTCCTGGCCCAACAACCATACGGTGACGGTGATGGTGCAAGTCCAGAATGTGGAACACC
GACCGCCGCGATGGATGGAAATCTTCGCAGTCCAGCAGTTTGACGAGATGACGGAGCAGCAATT
CCAGGTGCGCGCCATCGACGGAGACACTGGCATCGGGAAAGCTATACACTATACCCTCGAGACA
GATGAGGAAGAAGATTTGTTCTTCATCGAAACACTTCCGGGCGGCCATGACGGAGCCATCTTCA
GCACTGCCATGATTGATGTGGATAGGCTCCGGCGAGATGTCTTCAGACTGTCCCTGGTGGCATA
CAAGTACGACAATGTGTCCTTCGCCACCCCGACACCCGTCGTGATCATAGTCAATGACATCAAC
AACAAGAAACCCCAACCGCTGCAAGATGAGTACACAATCTCCATAATGGAAGAAACTCCACTGT
CGCTGAATTTTGCTGAACTTTTTGGTTTCTATGATGAAGATTTGATCTACGCACAATCCTTGGT
GGAAATACAAGGCGAGAACCCTCCAGGCGTAGAGCAAGCGTTTTATATTGCGCCCACCGCAGGC
TTCCAGAACCAGACATTCGCCATAGGGACTCAAGATCACCGAATGCTGGATTATGAGGATGTTC
CTTTCCAAAACATCAAGCTCAAGGTAATAGCAACGGACCGTGACAATACCAATTTTACTGGAGT
CGCGGAAGTCAACGTGAACCTGATTAATTGGAACGACGAGGAGCCGATCTTTGAGGAAGACCAG
CTCGTTGTCAAGTTCAAGGAGACTGTACCCAAGGACTATCACGTCGGCAGACTGAGGGCTCACG
ACCGGGACATAGGAGACAGCGTTGTGCATTCCATCTTGGGAAATGCGAATACATTTTTGAGAAT
CGACGAAGAAACTGGCGACATATACGTAGCTATTGATGACGCGTTCGATTATCACAGACAGAAT
GAATTTAACATACAAGTTCGCGCTCAGGACACCATGTCGGAGCCAGAGTCCAGGCATACAGCGG
CTGCTCAGCTGGTCATAGAACTCGAGGACGTCAACAACACACCTCCTACTCTGAGGCTGCCTCG
CGTAAGTCCGTCTGTAGAAGAGAATGTGCCAGAGGGCTTTGAAATCAACCGGGAGATAACCGCC
ACGGACCCTGACACCACAGCATACCTGCAGTTTGAAATAGATTGGGACACATCCTTTGCCACTA
AACAGGGGCGTGATACCAATCCAATAGAGTTCCACGGATGCGTGGATATAGAAACCATCTTCCC
AAACCCAGCCGACACCAGAGAGGCTGTGGGGCGAGTGGTAGCGAAGGGGATCCGCCATAACGTG
ACCATCCATTTTGAAGAGTTTGAATTTCTCTACCTCACAGTGAGAGTTCGGGACTTGCACACAG
ATGACGGACGAGATTATGATGAATCTACCTTCACGGTAATAATAATAGATATGAACGACAACTG
GCCTATCTGGGCGTCTGGTTTCCTGAACCAGACCTTCAGTATTCGGGAGCGATCATCTACCGGC
GTCGTCATCGGGTCCGTACTCGCTACAGACATTGATGGCCCACTTTACAACCAAGTCCGGTACA
CCATTATCCCCCAGGAAGATACTCCTGAAGGTCTAGTCCAGATACATTTCGTTACGGGTCAAAT
TACAGTTGATGAGAATGGTGCAATCGACGCTGATATTCCACCTCGTTGGCACCTCAACTACACG
GTTATAGCCAGCGACAAATGTTCTGAAGAAAATGAAGAGAACTGTCCCCCGGATCCAGTGTTCT
GGGATACTCTGCGCGACAATGTAATTAACATCGTGGACATAAACAACAAGGTCCCGGCAGCAGA
CCTCAGTCGATTCAACGAAACGGTGTACATTTATGAAAATGCACCCGATTTCACGAACGTGGTC

Figure 1A

AAGATATACTCCATCGACGAAGACAGAGACGAAATATATCACACGGTGCGGTACCAGATCAATT
ATGCTGTGAACCAACGGCTGCGAGACTTCTTCGCCATAGACCTGGATTCAGGCCAGGTGTACGT
GGAGAACACCAACAATGAGCTCCTGGATCGGGACAGAGGCGAAGACCAACACAGGATATTCATT
AACCTCATTGACAACTTTTATAGCGAAGGAGATGGAAATAGAAATGTAAACACTACAGAGGTGC
TGGTGATACTATTAGATGAGAATGACAACGCTCCTGAATTGCCGACTCCAGAAGAGCTGAGTTG
GAGCATTTCCGAGGATTTACAAGAGGGTATAACACTCGATGGCGAAAGCGATGTGATATACGCA
CCGGATATAGACAAAGAGGACACGCCAAACTCTCACGTTGGCTACGCAATCCTGGCCATGACAG
TCACCAATAGAGACCTGGACACTGTTCCGAGACTTCTCAACATGCTGTCGCCTAACAACGTAAC
CGGATTCCTCCAGACAGCAATGCCTTTGAGAGGATATTGGGGGACTTACGATATAAGTGTACTG
GCGTTCGACCACGGTATTCCTCAGCAGATATCTCATGAGGTGTATGAATTGGAAATTCGACCTT
ACAATTACAATCCTCCTCAGTTCGTTTTTCCTGAATCCGGGACGATTCTACGACTGGCTTTGGA
ACGCGCAGTGGTAAATAATGTTTTGTCACTTGTAAACGGTGACCCGTTAGACAGGATACAAGCA
ATTGACGACGATGGTCTTGATGCTGGCGTGGTGACTTTCGATATTGTTGGAGATGCTGATGCGT
CAAACTACTTCAGAGTAAATAATGATGGCGACAGCTTTGGAACCTTGTTGCTGACACAGGCGCT
TCCTGAGGAAGGCAAGGAATTTGAGGTTACCATCCGGGCTACAGACGGCGGAACAGAACCTCGA
TCATATTCAACAGACTCCACTATAACAGTCCTCTTCGTTCCGACTTTGGGTGATCCGATCTTTC
AAGATAACACTTACTCAGTAGCATTCTTTGAAAAAGAGGTTGGCTTGACTGAGAGGTTCTCGCT
CCCACATGCAGAGGACCCTAAGAACAAACTCTGCACTGACGACTGTCACGATATTTACTACAGG
ATCTTTGGTGGTGTGGATTACGAGCCATTTGACCTGGACCCGGTGACGAACGTGATCTTCCTGA
AATCAGAACTAGACCGGGAGACCACTGCTACGCATGTGGTGCAAGTGGCAGCCAGTAATTCGCC
CACAGGAGGCGGAATACCACTCCCTGGGTCTCTTCTCACCGTCACTGTCACTGTACGAGAAGCG
GATCCACGGCCTGTGTTCGAGCAGCGTCTGTACACGGCTGGCATTTCCACTTCCGATAACATCA
ACAGGGAACTACTCACCGTTCGTGCAACTCATTCCGAAAACGCACAATTGACATATACCATCGA
AGACGGTTCTATGGCGGTGGACTCCACTCTGGAAGCCGTCAAGGACTCGGCGTTCCATCTGAAC
GCGCAGACCGGCGTCCTCATACTGAGGATACAACCTACTGCCAGCATGCAGGGCATGTTTGAGT
TCAACGTCATCGCTACTGACCCAGATGAGAAGACAGATACGGCAGAGGTGAAAGTCTACCTCAT
TTCATCCCAAAATAGGGTGTCCTTCATATTCCTGAACGATGTGGAGACGGTTGAGAGTAACAGA
GACTTTATCGCAGAACGTTCAGCGTTGGCTTCAACATGACCTGCAATATAGATCAGGTGCTGC
CGGGCACCAACGACGCCGGGGTGATTCAGGAGGCCATGGCGGAAGTCCATGCTCACTTCATACA
GGATAACATCCCTGTGAGCGCCGACAGTATTGAAGAGCTTCGCAGTGACACTCAGCTGCTGCGC
TCCGTCCAAGGTGTGTTGAACCAACGGCTGTTGGTCCTGAACGACCTGGTGACGGGGGTCAGCC
CTGATCTCGGCACTGCCGGCGTGCAGATCACCATCTATGTGCTAGCCGGGTTGTCAGCCATCCT
TGCCTTCCTGTGCCTTATTCTGCTCATCACATTCATCGTGAGGACCCGAGCTCTGAACCGCCGT
TTGGAAGCACTGTCGATGACGAAATACGGCTCGGTGGATTCGGGGCTGAACCGAGTGGGGATAG
CGGCCCCAGGAACCAACAAACACGCCATCGAAGGCTCCAACCCCATCTGGAACGAGCAGATCAA
GGCCCCGGACTTCGATGCCATCAGTGACACATCTGACGACTCTGATCTAATCGGCATCGAGGAT
AGCCTGCAGGGAGACTTAGAAGAGAAAAGGGCAGACAAAGCAGTAGATGCCTTGGTGAAAAAGC
TGAAGAAGAACGATGGAGCCATGGGGGAATACGAATTCAAGGCCTCTCGAGCCTCTAGAACTAT
CGTGAGTCGTATTACGTATATCCAGACATGATGAGATACATTGATGAGTTTGGACAAACCGCAA
CTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGGAA
CCATTATAAGCTGCAATAAACAAGTTAACATCATCAATTGCATTCATTTTATGTTTCAGGTTCA
GGGGGAGGTGTGGGAGGCTATCC

Figure 1B

SIG

1 MAGDACILVT VLLTFATSVF GQETTSSRCY YMTDAIPREP KPDDLPDLEW

CR1 →

51 TGGWTDWPLI PAEPRDDVCI NGWYPQLTST SLGTIIIHME EEIEGDVAIA

101 KLNYDGSGTP EIVQPMVIGS SNLLSPEIRN ENGAWYLYIT NRQDYETPTN

CR2 →

151 RRYTFDVRVP DETRAARVSL SIENIDDNDP IVRVLDACQV PELGEPRLTD

201 CVYQVSDEDG RLSIEPMTFR LTSDREDVQI FYVEPAHITG DWFNMQITIG

CR3 →

251 ILSALNFESM PLEIPQITAL DSWFNNHTVT VMVQVQNVEH RPPRWMEIFA

301 VQQFDEMTEQ QFQVRAIDGD TGIGKAIEYT LETDEEEDLF FIETLPGGHD

351 GAIFSTAMID VDRLRRDVFR LSLVAYKYDN VSFATPTPVV IIVNDINNKK

CR4 →

401 PQPLQDEYTI SIMEETPLSL NFAELFGFYD EDLIYAQSLV EIQGENPPGV

451 EQAFYIAPTA GFQNQTFAIG TQDERMLDYE DVFFQNIKLK VIATDRQNTN

CR5 →

501 FTGVAEVNVN LINWNDEEFI FEEDQLVVKF KETVPKDYHV GRLRAHDRDI

551 GDSVVHSILG NANTFLRIDE ETGDIYVAID DAFDYHRQNE FNIQVRAQDT

CR6 →

601 MSEPESRHTA AAQLVIELED VNNTPFTLRL PRVSPSVEEN VPEGFEINRE

651 ITATDPDTTA YLQFEIDWDT SFATKQGRDT NFIEFHGCVD IETIFPNFAD

701 TREAVGRVVA KGIRPNVTIH FEEFEFLYLT VRVRDLETDD GRDYDESTFT

CR7 →

751 VIIIDMNDNW PIWASGFLNQ TFSIRERSST GVVIGSVLAT DIDGPLYNQV

801 RYTIIPQEDT PEGLVQIHFV TGQITVDENG AIDADIPPRW HLNYTVIASD

CR8 →

851 KCSEENEENC PPDPVFWDTL RDNVINIVDI NNKVPAADLS RPNETVYIYE

901 NAPDFTNVVK IYSIDEDRDE IYHTVRYQIN YAVNQELRDF FAIDLDSGQV

951 YVENTNNELL DRDRGEDQHR IFINLIDNFY SEGDGNRNVN TTEVLVILLD

CR9 →

1001 ENDNAPELPT PEELSWSISE DLQEGITLDG ESDVIYAPDI DKEDTPNSHV

1051 GYAILAMTVT NRDLDTVPRL LNMLSPNNVT GFLQTAMPLR GYWGTYDISV

1101 LAFDHGIPQQ ISHEVYELEI RPYNYNPPQF VFPESGTILR LALERAVVNN

Figure 2A

CR10 →

1151 VLSLVNGDPL DRIQAIDDDG LDAGVVTFDI VGDADASNYF RVNNDGDSFG

1201 TLLLTQALPE EGKEFEVTIR ATDGGTEPRS YSTDSTITVL FVPTLGDPIF

CR11→ MBF

1251 QDNTYSVAFF EKEVGLTERF SLPHSEDPKN KLCTDDCHDI YYRIFGGVDY

1301 EPFDLDPVTN VIFLKSELDR ETTATHVVQV AASNSPTGGG IPLPGSLLTV

CR12→

1351 TVTVREADPR PVFEQRLYTA GISTSDNINR ELLTVRATHS ENAQLTYTIE

1401 DGSMAVDSTL EAVKDSAFHL NAQTGVLILR IQPTASMQGM FEFNVIATDP

→ MPD

1451 DEKTDTAEVK VYLISSQNRV SFIFLNDVET VESWRDFIAE TFSVGFNMTC

LZ

1501 NIDQVLPGTN DAGVIQEAMA EVHAHFIQDN IPVSADSIEE LRSDTQLLRS

1551 VQGVLNQRLL VLNDLVTGVS PDLGTAGVQI TIYVLAGLSA ILAFLCLILL

→ CYT

1601 ITFIVRTRAL NRRLEALSMT KYGSVDSGLN RVGIAAPGTN KHAIEGSNPI

1651 WNEQIKAPDF DAISDTSDDS DLIGIEDSLQ GDLEEKRADK AVDALVKKLK

1701 KNDGAMGEYE FKASRASRTI VSRITYIQT.

Figure 2B

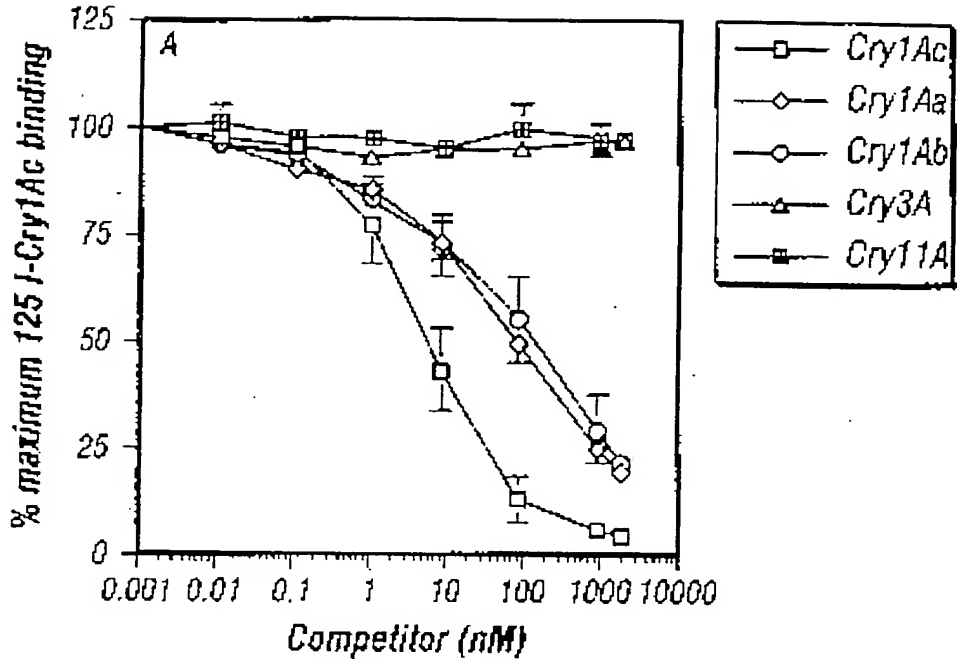

A
% maximum 125 I-Cry1Ac binding
125
100
75
50
25
0
0.001 0.01 0.1 1 10 100 1000 10000
Competitor (nM)
Cry1Ac
Cry1Aa
Cry1Ab
Cry3A
Cry11A

FIG. 3A

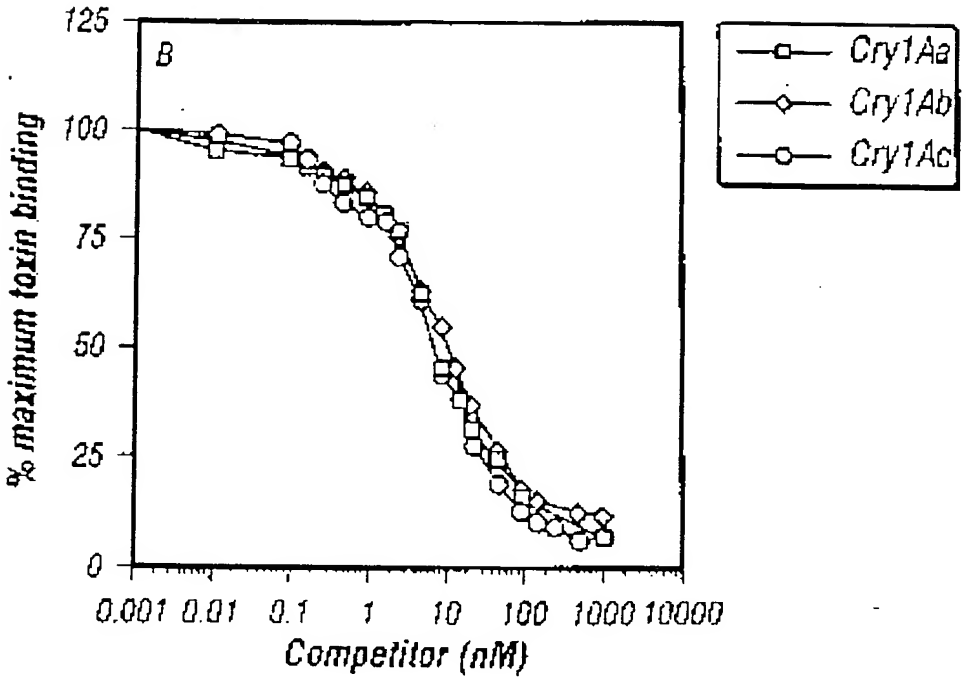

B
% maximum toxin binding
125
100
75
50
25
0
0.001 0.01 0.1 1 10 100 1000 10000
Competitor (nM)
Cry1Aa
Cry1Ab
Cry1Ac

FIG. 3B

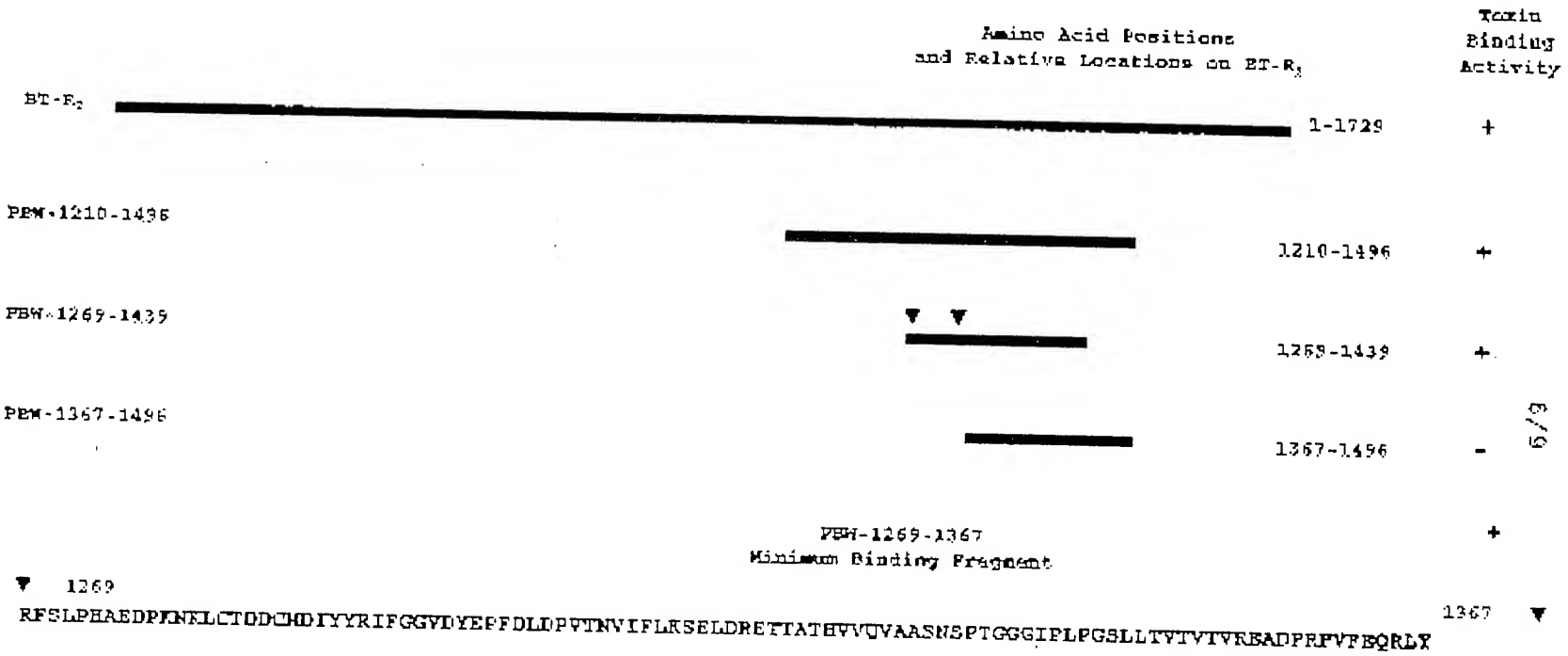

Amino Acid Positions
and Relative Locations on ET-R1
Toxin
Binding
Activity
ET-R2
1-1729
+
PEW-1210-1496
1210-1496
+
PEW-1269-1439
1269-1439
+
PEW-1367-1496
1367-1496
-
PEW-1269-1367
Minimum Binding Fragment
+
1269
1367
RFSLPHAEDPFINELCTDDCHDIYYRIFGGVDYEPFDLDPVTNVIFLRSELDRETTATHVVCVAASNSPTGGGIPLPGSLLTVTVTVRSADPRPVTEQRLY

Figure 4

# Figure 5A

# Figure 5B

# Figure 5C